

SPECIFIC RECRUITMENT OF STRONGLY DIFFERENTIATED CD8+ T CELLS IN THE CEREBROSPINAL FLUID OF PATIENTS WITH MULTIPLE SCLEROSIS

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INTRODUCTION:

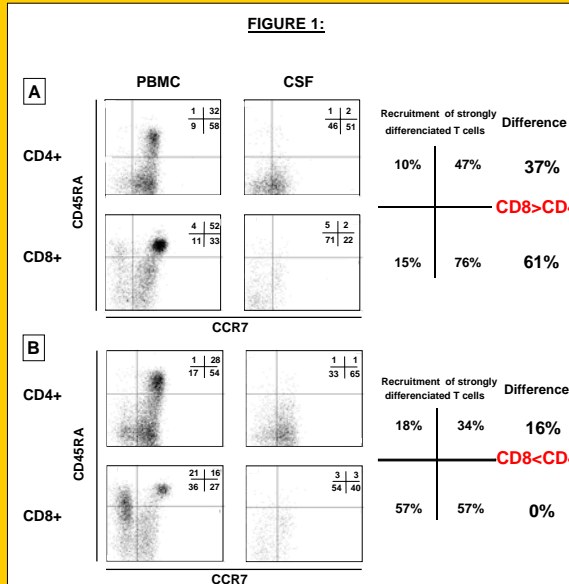
Recent observations have suggested that CD8+ T cells might play a more important role in the pathogenesis of MS than previously thought. Novel studies on the EAE animal model have shown that autoreactive CD8+ T cells produced a severe form of EAE [1,2]. Lesions in this model are located mostly in the brain rather than in the spinal cord, contrary to what is seen in most CD4+ T cell-associated EAE models. In this regard, the EAE CD8+ T cell model is more similar to human MS than the EAE CD4+ T cell model.

In humans, CD8+ T cells are more abundant in MS lesions than CD4+ T cells [3]. This numerical dominance in all kinds of inflamed CNS tissue suggests that migration into and homing in the CNS is facilitated for class I compared with class II restricted T lymphocytes [4]. Indeed *in vitro* and *in vivo* experiments have shown that all types of CNS cells, including neurons and oligodendrocytes, can express MHC class I molecules, especially under inflammatory conditions. There is an oligoclonal expansion of CD8+ T cells in the CSF and in the white matter lesions of MS patients [5,6]. Taken together, these data suggest that an antigen-specific and highly focused cellular immune response - mediated by CD8+ T cells - takes place. However, the phenotype of CD8+ versus CD4+ T cells remains unknown. In addition, if CD8+ T cells play a role, one can hypothesize that they do so in the very early stages of MS.

In this study, we examined the profile of the immune response in the blood and in the cerebrospinal fluid (CSF) of patients with suspected MS (Su-MS). These patients typically presented with a first episode consistent with MS. Alternatively, they had already had previous neurological deficits, but a diagnosis of MS had not been established yet. A lumbar puncture (LP) was performed as part of the diagnostic work-up. To study the degree of differentiation of CD4+ and CD8+ T lymphocytes in the blood and the CSF, we used the combination of the chemokine receptor CCR7 and the human leukocyte common antigen CD45RA [7].

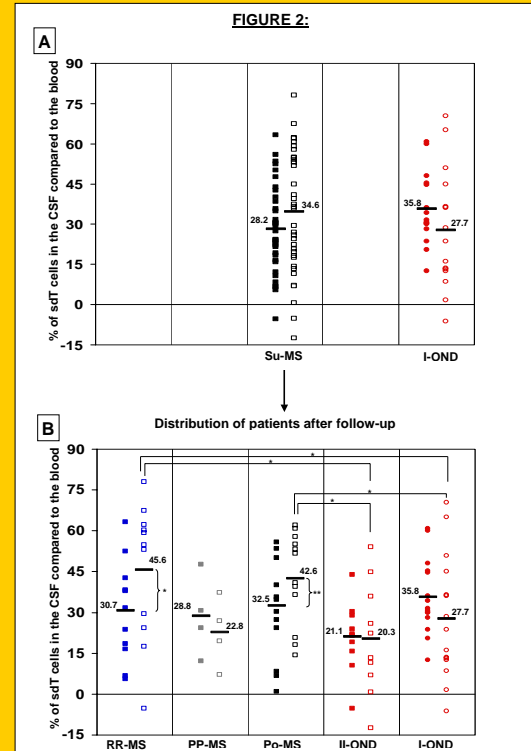
REFERENCES:

1. Sun et al., J Immunol 2001;166.
2. Huseby et al., J Exp. Med 2001;194.
3. Gay et al., Brain 1997;120.
4. Bauer et al., Glia 2001;36.
5. Babbe et al., J Exp Med 2000;152.
6. Jacobsen et al., Brain 2002;125.
7. Champagne et al., Nature 2002;401.



Comparison of the phenotype of T cells between the blood and the CSF compartments in two representative patients, one with RR-MS and one with OND. These analyses were performed on CD3+CD8+ and CD3+CD8- T cells, the latter considered to be CD4+ T cells. The phenotype of these two subtypes of T cells was determined by staining them with CCR7 (X-axis) and CD45RA (Y-axis). Cells in the upper right quadrant correspond to naive T cells; in the lower right quadrant to central memory T cells; in the lower left quadrant to effector memory T cells; and in the upper left quadrant to effector T cells [7]. The percentage of T cells in each quadrant is indicated. Strongly differentiated T cells (sdT cells) correspond to the sum of the percentage of effector memory (CCR7-/CD45RA-) and effector T cells (CCR7-/CD45RA+). Exactly the same gating was applied between the blood and CSF compartment. CD3+ was coupled to PerCP-Cy5.5; CD8+ to APC; CCR7 to FITC and CD45RA to PE fluorescent antibodies.

- A).** In this RR-MS patient, the percentage of strongly differentiated CD4+ T cells (sdCD4+) increased between the blood (10%) and the CSF (47%) compartment (difference: 37%) but the percentage of sdCD8+ T cells in the CSF (76%) compared to the blood (15%) was higher (difference 61%). In other terms, the recruitment of sdCD8+ T cells was higher than the one of sdCD4+ T cells in this patient and the difference was: 61%-37% = 24% in favor of sdCD8+ T cells.
- B).** For this patient with OND (cervical discal protrusion), the picture was opposite: the recruitment of sdCD4+ T cells in the CSF compared to the blood (difference: 16%) was higher than the one of sdCD8+ T cells (difference: 0%).



Higher recruitment of CD8+ T cells in the CSF of patients who will develop MS than those who will develop an OND.

We enrolled 52 patient who had to undergo a lumbar puncture (LP). At the time of the LP, there were two categories of patients: 37 with suspected MS (Su-MS) and 15 with an OND from the outset (I-OND). The difference of percentage of sdT cells in the CSF compared to the blood for CD4+ (closed symbols) and CD8+ (open symbols) T cells is represented.

B). Patients were prospectively followed from 1 to 16 months (mean 9.7 +/- 4.4). Among the 37 Su-MS, 15 developed definite MS, including 11 with relapsing-remitting (RR-MS) and 4 with primary-progressive (PP-MS); 10 turned out to have an OND (I-OND); and 12 still have a diagnosis of possible MS (Po-MS).

Statistics: differences in CSF recruitment of sdCD4+ vs sdCD8+ T cells in a same category of patients was calculated using the Wilcoxon signed ranked test (braces). Differences of CSF recruitment for a subtype of T cells between two categories of patients was performed with the Mann Whitney test (bars). Only significant results are shown. * p < 0.05; **p < 0.01

TABLE: CSF humoral and cellular markers

	Recruitment of sdCD8+ > sdCD4+ T cells in the CSF	Intra-thecal synthesis of IgG (IS IgG)	Both
RR-MS	9/11	11/11	9/11 (82%)
PP-MS	0/4	4/4	0/4 (0%)
Po-MS	9/12	7/12	6/12 (50%)
OND total (I+II)	9/25	4/25	1/25 (4%)

Nine of eleven patients with Su-MS who had a recruitment of sdCD8+>sdCD4+ T cells in the CSF developed MS versus 9/25 patients with an OND, a difference found to be significant: p= 0.028, Fisher exact test. In parallel, 11/11 Su-MS patients who were prone to develop MS had an IS IgG versus 4/25 with an OND (p<0.0001).

The combination of an IS IgG and a CSF recruitment of sdCD8+>sdCD4+ T cells might reinforce the likelihood of MS. Indeed, 9/11 with RR-MS had such a combination versus only 1/25 (p<0.0001). In other terms, when these two markers are present in a given patient with Su-MS, it is extremely unlikely that he will develop an OND.

Interestingly, a recruitment of sdCD8+>sdCD4+ T cells does not appear to be present in PP-MS, whereas these patients do have an IS Ig. Therefore, the combination of these two markers might help to distinguish, early on, which form of MS the patient will develop.

Finally, 6/12 patients with Po-MS have both markers. Based on our data, we hypothesize that these 6 patients will develop MS.

CONCLUSION:

- There is a higher recruitment of strongly differentiated (sd) CD8+ than CD4+ T cells in the CSF of patients with MS.
- This high recruitment of sdCD8+ T cells is already present in the early stages of MS (first relapse), and seems to be correlated to the inflammatory stages of MS.
- Our study tends to confirm the fact that CD8+ T cells play an important role in the physiopathology of MS.
- The higher recruitment of sdCD8+ vs sdCD4+ T cells in the CSF combined with the detection of an intra-thecal synthesis of IgG could be a useful set of markers to establish a diagnosis of MS earlier in the course of the disease.

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