

Enrichment of strongly differentiated CD8+ T cells in the CSF of patients with early multiple sclerosis

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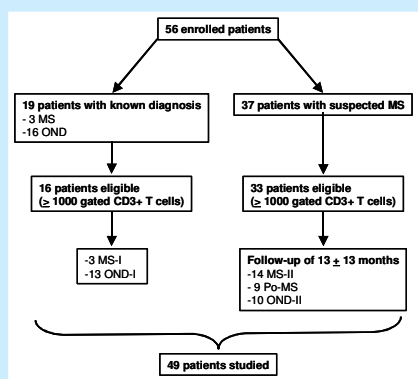
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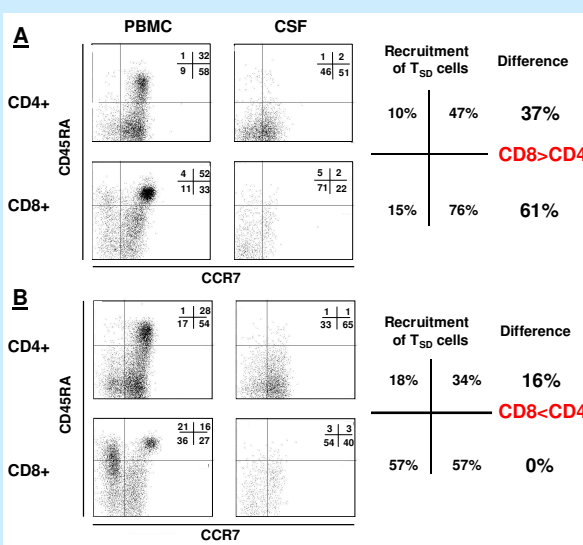
INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disease which leads to demyelination of the central nervous system [1]. CD4+ T cells have long been thought to play an important role in the immunopathogenesis of this disease [2]. In particular, this role was suggested by the data obtained in a MS animal model, the experimental autoimmune encephalomyelitis (EAE) [3]. Although considered for long as irrelevant in MS, CD8+ T cells have recently gained a renewed interest as a potential important player in MS [4,5,6]. However, the respective role of these cells early in the course of the MS is poorly understood.

Here, we aimed at characterizing CD4+ and CD8+ T cells in patients with MS, in particular those who presented with a first neurological episode consistent with MS. We examined the profile of the immune response in the blood and in the cerebrospinal fluid (CSF) of these patients. They typically presented with a first episode consistent with MS, but a diagnosis of MS had not been established yet. Patients with other neurological diseases (OND) were used as controls. A lumbar puncture (LP) was performed as part of the diagnostic work-up. To study the degree of differentiation of CD4+ and CD8+ T cells in the blood and the CSF, we used the combination of the chemokine receptor CCR7 and the human leukocyte common antigen CD45RA [7].



Study design. Fifty-six patients undergoing a LP were enrolled. Nineteen had a known diagnosis, 37 had a first episode consistent with MS. PBMC and CSF cells were stained with CD3, CD8, CCR7 and CD45RA markers. Patients eligible for the study had at least 1000 CD3+ gated T cells in their CSF. During the follow-up period (13±13 months), 14 patients turned out to have MS, 10 patients had OND and nine patients still have a diagnosis of Po-MS.

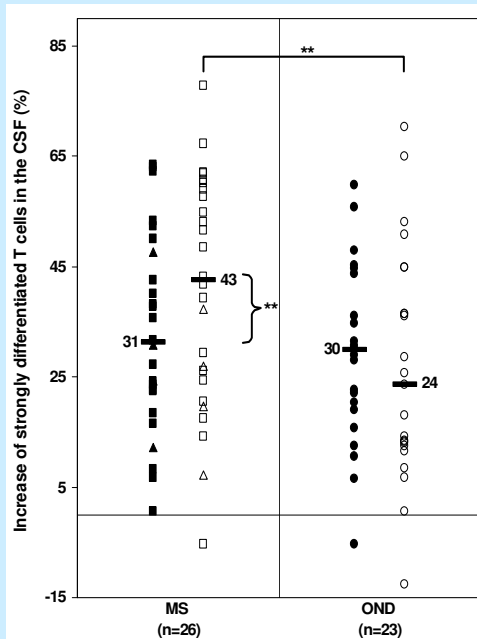


Comparison of the phenotype of T cells between the blood and the CSF compartments in two representative patients, one with RR-MS and one with OND.

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 with CCR7 (X-axis) and CD45RA (Y-axis). Cells in the upper right quadrant correspond to naive T cells (T_N); in the lower right quadrant to central memory T cells (T_{CM}); in the lower left quadrant to effector memory T cells (T_{EM}); and in the upper left quadrant to effector T cells (T_E) [8]. The percentage of T cells in each quadrant is indicated. Strongly differentiated T cells (T_{SD} cells) correspond to the sum of the percentage of T_{EM} and T_E. Exactly the same gating was applied between the blood and CSF compartment.

A). In this RR-MS patient, the percentage of CD4+ T_{SD} cells increased between the blood (10%) and the CSF (47%) compartment (difference: 37%) but the percentage of CD8+ T_{SD} cells in the CSF (76%) compared to the blood (15%) was higher (difference 61%). In other terms, the recruitment of CD8+ T_{SD} cells was higher than the one of CD4+ T_{SD} cells in this patient and the difference was: 61%-37% = 24% in favor of CD8+ T_{SD} cells.

B). For this patient with non-inflammatory OND, the picture was opposite: the recruitment of CD4+ T_{SD} cells in the CSF compared to the blood (difference: 16%) was higher than the one of CD8+ T_{SD} cells (difference: 0%).



Higher recruitment of strongly differentiated CD8+ T cells in the CSF of patients with MS than in those with OND. The determination of T cells phenotype was performed on paired samples of peripheral blood and CSF at the time of the lumbar puncture. Each symbol corresponds to difference in percentage, between peripheral blood and CSF of T_{SD} cells for CD4+ (closed symbol) and CD8+ T cells (open symbol). Thus, there are two symbols per patient (one close and one open).

** p<0.03 (MS CD8+ vs OND CD8+, Mann-Whitney test; CD4+ vs CD8+, Wilcoxon test)

□, ■: RR-MS and SP-MS; ▲, △: PP-MS; ○, ●: OND

The CSF enrichment in CD8+ T_{SD} cells was significantly higher in MS than in OND. No such difference existed for CD4+ T_{SD} cells between MS and OND patients. Furthermore the enrichment in CD8+ T_{SD} cells was significantly higher than the one in CD4+ T_{SD} cells in MS patients. Such a difference was absent in OND patients.

	RRMS-II (n=9) SPMS-II (n=2) Po-MS (n=9)	OND-II (n=10) PP-MS (n=3)
intrathecal synthesis of IgG	19	4
no intrathecal synthesis of IgG	1	9
N = 33		

	RRMS-II (n=9) SPMS-II (n=2) Po-MS (n=9)	OND-II (n=10) PP-MS (n=3)
CD8/CD4 T _{SD} cells >1	14	0
CD8/CD4 T _{SD} cells <1	5	4
N = 23		

Sensitivity: 19/19+1 = 19/20 = 95%
Specificity: 9/9+4 = 9/13 = 69%
Positive predictive value: 19/19+4 = 19/23 = 83%
Negative predictive value: 9/9+1 = 9/10 = 90%

Sensitivity: 14/14+5 = 14/19 = 74%
Specificity: 4/4+0 = 4/4 = 100%
Positive predictive value: 14/14+0 = 14/14 = 100%
Negative predictive value: 4/4+5 = 4/9 = 44%

Among patients with suspected MS who have an intrathecal synthesis of IgG (ISIgG+), the ratio of CSF enrichment of CD8+ versus CD4+ T_{SD} cells helps to discriminate between PP-MS and OND patients on one side from patients with other types of MS on the other side.

Left panel: Distribution of the 33 patients with Su-MS according to their final diagnosis. The sensitivity, specificity, positive predictive value, and negative predictive value of an ISIgG+ for the diagnosis of MS are displayed.

Right panel: The 23 Su-MS patients with an ISIgG+ are sorted according to their final diagnosis and the CSF enrichment ratio of CD8/CD4 T_{SD} cells. Only patients with RR-, SP-, or Po-MS, but not PP-MS or OND, had both an ISIgG+ and a CSF enrichment ratio of CD8/CD4 T_{SD} cells >1 (specificity and positive predictive value = 100%). However, as reflected by the low negative predictive value of this test, a CSF enrichment ratio of CD8/CD4 T_{SD} cells <1 does not allow to rule out RR-, SP- or Po-MS. CD8/CD4 T_{SD} cells, ratio of CSF enrichment of CD8+ compared to CD4+ T_{SD} cells.

CONCLUSION

- There is a higher enrichment in strongly differentiated CD8+ T cells than in CD4+ T cells in the CSF of patients with early MS.
- The enrichment in strongly differentiated CD8+ T cells is particularly high in patients with early MS.
- Our study tends to confirm the fact that CD8+ T cells play an important role in the immunopathogenesis of MS.
- Combination of an intrathecal synthesis of IgG and a ratio of enrichment of strongly differentiated CD8/CD4 T_{SD} cells > 1 might improve the diagnosis of certain forms of MS, RR-MS in particular.

REFERENCES

- Noseworthy et al. (2000) *N Engl J Med* 343:938
- O'Connor et al. (2001) *J Clin Immunol* 21:81
- Bischof et al. (2004) *J Immunol* 172:2878
- Gay et al. (1997) *Brain* 120:1461
- Bitsch et al. (2000) *Brain* 123:1174
- Neumann et al. (2002) *Trends Neurosci* 25:313
- Champagne et al. (2002) *Nature* 410:106

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