

Highly differentiated CD8+ T cells have different characteristics in the CSF than in the blood

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INTRODUCTION:

Although considered for long as irrelevant in MS, CD8+ T cells have recently gained a renewed interest as a potential important player in MS [1,2,3]. However, the respective role of these cells early in the course of the MS is poorly understood.

We have previously shown that highly differentiated CD4+ and CD8+ T cells (effector memory and effector cells) were recruited in the CSF of patients with MS or with other neurological diseases (OND). Patients with early relapsing-remitting (RR-) MS had a greater enrichment in highly differentiated CD8+ T cells (CD8+ T_{HD}) than CD4+ T_{HD} cells. CD8+ T_{HD} cells were also more enriched in the CSF of early RR-MS than in the CSF of primary-progressive (PP-) MS or OND patients. These findings suggest a role for this subset of T cells at the onset of the disease [4].

In this study, we wanted to characterize further these T_{HD} cells in the PBMC and CSF of MS and OND patients, using CD28, a co-stimulatory molecule important for the activation of T cells; perforin, a marker of cytotoxic T cells; and FOXP3, a marker of regulatory T cells.

MATERIAL AND METHODS:

Patients: We enrolled patients with possible or definite RR-MS (RR-Po-MS) and patients with OND at the outpatient clinics. All patients gave their informed consent according to the IRB of our hospital. PBMC and CSF (when available) were obtained and immediately processed.

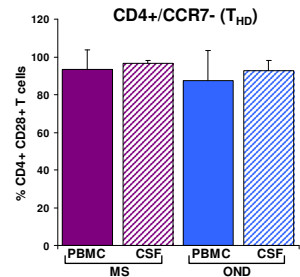
Staining: Cells were stained using different combinations for CD3, CD4, CD8, CCR7, CD45RA, CD28, perforin and FOXP3. Following antibodies were used: CD3-PE, CD4-APC, CD4-PerCP-Cy5.5, CD8-PerCP, CD8-Pacific Blue, CCR7-FITC, CD45RA-PE, CD28-APC, perforin-FITC, FOXP3-PE.

Flow cytometry: Cells were analysed by 6- and 7-parameters flow cytometry on a FACSCalibur and LSR II.

Statistics: Wilcoxon signed ranked test and Mann-Whitney test were used.

ACKNOWLEDGEMENTS:

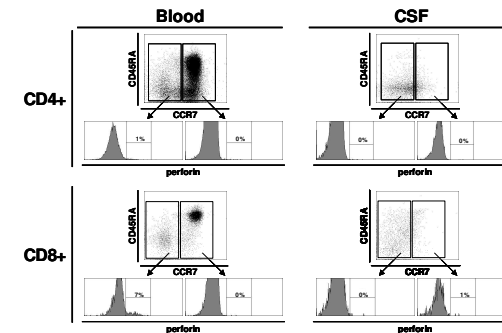
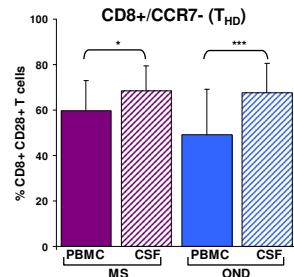
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CD28 expression in CD4+ and CD8+ T_{HD} cells (CCR7-) in PBMC and CSF of RR-/Po-MS and OND patients. Using flow cytometry, CD28 expression was assessed on CCR7- T_{HD} cells, either CD4+ or CD8+ in the blood and the CSF. There were 17 patients with possible MS or definite RR-MS (MS; purple) and 24 with OND (blue). Bars represent mean; error bars, SD. Differences of CD28 expression between PBMC and CSF was calculated using the Wilcoxon signed ranked test. Differences of CD28 expression between the two categories of patients was performed with the Mann-Whitney test. * p<0.05; *** p<0.005

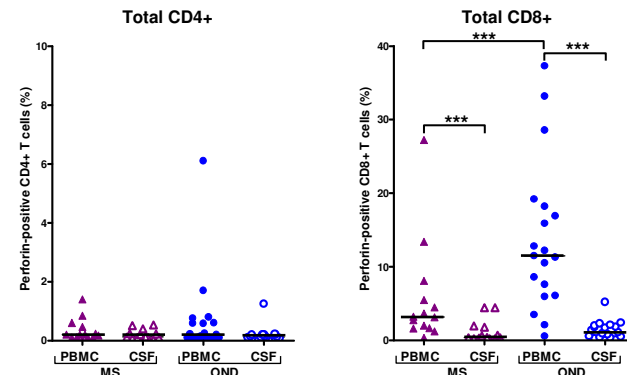
Left panel: CD28 expression was highly prevalent on CD4+ T_{HD} cells without difference between PBMC and CSF.

Right panel: By contrast, there was a significant lower expression of CD28 on CD8+ T_{HD} cells in the blood as compared to the CSF. These results suggest that CD8+ T_{HD} cells that were recruited in the CSF were less activated than in the blood.



Perforin-positive cells are found in the CCR7- T_{HD} cell populations. Concomitant expression of perforin, CCR7 and CD45RA was assessed in the PBMC and the CSF of the patients. A representative example of a RR-MS patient is shown here.

These data show, that the few CD8+ T cells that contained perforin usually were highly differentiated, as revealed by the loss of CCR7 expression. These data are consistent with what was shown for virus-specific CD8+ T cells [5,6]. No perforin was detected in highly differentiated CD4+ T cells, which confirms that these cells are not cytotoxic T cells.



Perforin content in total CD4+ and CD8+ T cells in the PBMC and the CSF of RR-/Po-MS versus OND patients.

Expression of perforin in CD4+ and CD8+ T cells was assessed in the PBMC and the CSF of possible MS and definite RR-MS (MS; n=14, purple) and OND (n=19, blue) patients. Each symbol corresponds to the percentage of perforin in the blood (closed symbols) and in the CSF (open symbols). Horizontal bars represent the median values. Δ, ▲ MS; ○, ● OND. Differences of perforin content between PBMC and CSF was calculated using the Wilcoxon signed ranked test. Differences of perforin content between the two categories of patients was performed with the Mann-Whitney test. *** p<0.005.

CD8+ T cells in the blood of OND contained a higher amount of perforin than those of RR-/Po-MS patients. Perforin content decreased significantly in the CSF cells for all groups. These results showing that CD8+ T cells from MS patients did not contain a particularly high amount of perforin suggest that, in their majority, they were not highly activated cytotoxic T lymphocytes.

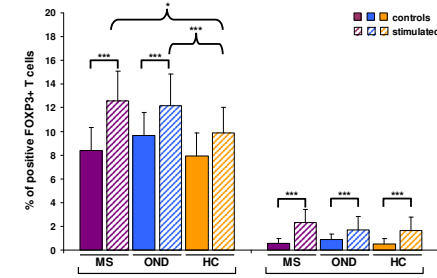
CONCLUSION:

We have previously shown that there was a recruitment in CD8+ T_{HD} cells in the CSF of MS patients [4]. We hypothesized that these cells were highly activated (CD28-), perforin containing CD8+ T cells. However, somewhat to our surprise, CD8+ T_{HD} cells in the CSF do not seem to lose CD28 expression as much as in the PBMC. These results suggest that they are not as much activated as in the PBMC. In accordance with this observation, they contain little perforin.

Thus in a second step, we wanted to determine whether these CD8+ T_{HD} cells were rather CD8 suppressor T cells. Indeed, it was recently shown that some CD8+CD28- T cells are able to suppress CD4+ T cell responses either through soluble factors, such as IL-10, or through down-regulation of co-stimulatory molecules at the surface of antigen presenting cells [7]. Interestingly, patients with autoimmune disease, including multiple sclerosis, were shown to have a decreased amount of CD8+ T suppressor cells in the blood [8,9]. Furthermore, some authors have suggested that suppressor CD8+ T cells might be of crucial importance in controlling relapses in MS [10,11].

Therefore, in an attempt to detect putative regulator/suppressor T cells in the PBMC of our patients, we looked at the expression of FOXP3 in the PBMC of our patients. We found that CD4+ T cells of MS and OND patients had a higher basal expression of FOXP3 as well as a higher increase in expression upon specific stimulation than HC. However, in contrast to previous studies [8,11], no difference in FOXP3 expression was found for CD8+ T cells between the different categories of patients.

In conclusion, CD8+ T_{HD} cells in MS do not exhibit particular cytotoxic or suppressor characteristics, at least as evaluated by perforin and FOXP3. In addition, despite the fact that they are highly differentiated, these cells seem less activated than their counterparts in the blood, as shown by CD28 expression. Additional markers are necessary to better characterise the nature of these cells in the CSF.



Non-specific stimulation of T cells with anti-CD3 induces upregulation of FOXP3 in CD4+ and CD8+ T cells in the PBMC.

We enrolled 30 patients: 20 with Po-MS or definite RR-MS (MS, purple), 10 with OND (blue) and 13 healthy volunteers (HC, orange). PBMC were stimulated for 18 hours with anti-CD3 and stained for CD4, CD8 and FOXP3.

Bars represent mean; error bars, SD. Differences in FOXP3 expression in a same category of patients was calculated using the Wilcoxon signed ranked test (braces). Differences in FOXP3 expression 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<0.005

Specific stimulation with anti-CD3 induced a higher expression of FOXP3 in CD4+ T cells of RR-/Po-MS and OND patients than of HC. The same specific stimulation enhanced the expression of FOXP3 in CD8+ T cells of all three categories of patients. Of note, CD28 expression on CD4+ and CD8+ T cells did not change after anti-CD3 stimulation (results not shown).

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