

" Analysis of CD4+RUNX3+ T lymphocytes during clinical course of experimental autoimmune encephalomyelitis ".

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Abstract

During the maturation of T lymphocytes in the thymus the balance in between the Runx3 and ThPOK expression are essential for the differentiation of the T CD4+CD8+ lymphocytes into T single positive lymphocytes CD4+ or CD8+. The expression of ThPOK along with GATA3 favors the commitments of immature thymocytes with the T CD4+CD8- lineage. On the other hand, the expression of Runx3 inhibits the expression of ThPOK favors the T CD4+CD8- lineage. However, during the study of T lymphocytes in animals with EAE, we identified that the T CD4 + autoreactive lymphocytes start expressing Runx3, especially when infiltrated into the CNS. Besides, we observed that these cells increase the expression of Granzyme B and CD4 + cells originally were expressing only IL-17 (Th17) are also expressing IFN- γ . Thus, with this project, we intend to help elucidate the evolution of autoreactive T cell profile during the course of EAE.

Key words:

EAE, Runx3, T CD4 lymphocytes

Introduction

Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) that results from an autoregressive response against the myelin sheath components. Much of knowledge gathered about the mechanism involved in the inflammatory processes of MS in the last decades is due to studies conducted on the experimental model, the Experimental autoimmune encephalomyelitis (EAE). EAE is considered a disease mediated by T CD4 lymphocytes, although it isn't clear how cells with functions that are mainly *helper* can initiate the inflammatory process that culminates in demyelinating. We observed that a percentage of the T CD4 lymphocytes present in the central nervous system (CNS) exhibits a cytotoxic or double positive (CD4 \cdot CD8 \cdot) profile. Besides, we noticed that these cells express Runx3 and IFN γ . The determination of the cell-fate of T lymphocytes in the Thymus is done by the balance of Thpok and Runx3, that are responsible for inducing the DP lymphocytes differentiation into single positive CD4 \cdot or CD8 \cdot ^{1,2} lymphocytes, respectively. These transcription factors have suppressive action over each other: the Runx3 expression inhibits the CD4 gene expression in the DP cells, making them obtain cytotoxic CD8 \cdot phenotype, thus leave the Thymus and go to the periphery of the immune system. Thus, the Runx3 differential expression could explain the cytotoxic profile of the T CD4 \cdot lymphocytes, along with the existence of T CD4 \cdot CD8 \cdot cells in the periphery.

Results and Discussion

Our results showed that the genic and protein expressions of Runx3 are increased in the TCD4+ lymphocytes infiltrated in CNS during the course of EAE. Besides, we were able to evidence that the Thpok expression doesn't change in both conditions, this keeps constant. Compatibly with the increase of Runx3, there are increase of Granzyme B, that expression traditionally is associate to TCD8+ cells. We observed to that the expression of Runx3 is increased in cells with Th1 profile, suggesting that the expression of Runx3 shall induce the

expression of Granzyme B, IFN- γ and CD8 in encephalitogenic CD4+ T lymphocytes.

Conclusions

Given the above, our data indicate that the expression of Runx3 is the pivotal cause of these phenotypic changes observed in the TCD4 cells during the clinical course of EAE. The appearance of the cytotoxic profile in TCD4 cells help to explain, at least parts, how cells considered helpers are succeed to have a effector action to overcome the hematoencephalic barrier and start the damages in CNS. The expression of the CD8 molecule into mature cells that expressed only CD4 (becoming CD4+CD8+) seems to be just another consequence of the Runx3 expression. Similarly, the expression of IFN γ follows this Runx3 expression, what justifies the subtype switch from Th17 to Th1 already reported by other groups^{3,4}.

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