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Evaluation of the cytotoxic profile of CD4 + T cells in the spontaneous autoimmune diabetes model in NOD (Non Obese Diabetic) mice.

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Abstract
Type 1 Diabetes Mellitus is an autoimmune disease characterized by the destruction of the pancreatic beta cells by the action of autoreactive T cells. Using Non Obese Diabetic (NOD) mice, expression of cytotoxicity-related molecules on CD4 + helper T cells in the pancreas and peripancreatic lymph node of diabetic animals was observed. However, unlike what has been seen in the EAE model, the expression of one of the major cytotoxic molecules is similar in both organs, while two others express much more in the target organ of diabetes than in the draining lymph node.

Key words:
Diabetes Type 1, T CD4+ Cell, Cytotoxicity.

Introduction

Autoimmune Type 1 Diabetes (DM1A) is one of the most common chronic diseases of childhood and adolescence, being characterized as an autoimmune disease and by the extensive destruction of the pancreatic beta cells by autoreactive CD4+ and CD8+ T lymphocytes1. Results from our group suggest that CD4+ T lymphocytes acquire a cytotoxic phenotype in the multiple sclerosis model, which, like the spontaneous diabetes model in NOD, are models of tissue-specific autoimmunities mediated by CD4+ T cells. Thus, it was our intention in the present proposal to evaluate if this cytotoxic profile found in autoaggressive CD4+ T lymphocytes is repeated in another autoimmune pathology that does not present the nervous system as a target organ. Therefore, we chose to work with spontaneous Diabetes Mellitus in NOD animals.

Results and Discussion

The progressive increase in the glycemia of mice indicates that the infiltration of lymphocytes in the pancreas occurs normally and, therefore, diabetes is developing.
We selected pre-diabetic and healthy animals for the acquisition of CD4+ T lymphocytes from the peripancreatic lymph nodes and pancreas. The samples were then submitted to qPCR analysis for the expression of molecules related to cytotoxic activity or to inflammatory response. The diabetic animals presented a higher mRNA expression of Runx3 and GzmB in the CD4 + T lymphocytes, both in the lymph nodes and infiltrates in the pancreas, in comparison with healthy animals. For the rest of the molecules, new analyzes should be made using a pre-amplification kit since the low amounts of cells obtained in the cell sorting.
In order to extend the analysis of the expression of cytotoxic response molecules in CD4 + T lymphocytes, we evaluated the expression of some of these molecules by flow cytometry. For this, we analyzed the expression of these cytotoxic molecules in CD3+CD4+ lymphocytes from peripancreatic lymph nodes and pancreas from diabetic and pre-diabetic animals. Our results demonstrated that there is no difference in the expression of these molecules when comparing the two groups, especially in pancreatic infiltrated CD4+ T cells. In lymph nodes we found a higher expression of Eomes, and a lower expression of CD134 in diabetic animals compared to pre-diabetic animals.
Eomes is a transcription factor related to cytotoxic activity, especially in the induction of GzmB expression. However, despite the greater expression of Eomes, the expression of GzmB does not show great change. The lower expression of CD134 could, to a certain extent, be related to the greater progression of inflammatory activity in diabetic animals. CD134 is a coestimulatory molecule that would also be related to the activation of the cytotoxic program of both CD4+ and CD8+ T lymphocytes. Thus, it is possible that the progression of the inflammatory response in diabetic animals is already at a more advanced stage and that the expression of these molecules is no longer essential for the construction of the effector profile.

Conclusions

Our data show that CD4 + T lymphocytes develop a cytotoxic profile during the clinical evolution of the disease. Yet, our preliminary data indicate that the construction of this cytotoxic profile is a common event in autoimmune diseases mediated by CD4+ T cells. Interestingly, the cells present in the peripancreatic lymph nodes exhibit granzyme B expression comparable to the same cell type infiltrated in the pancreas. In addition, our data demonstrate increased expression of Runx3 and GzmB B in the lymph node of diabetic animals compared to healthy controls.

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