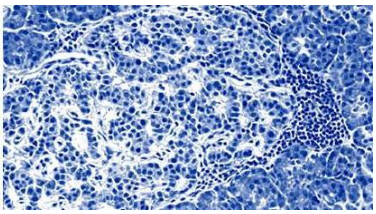
nPOD
Network for Pancreatic Organ Donors with Diabetes



A role for Deaf1 in the regulation of cytotoxic/NK genes in Type 1 diabetes?

AUTHORS

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PURPOSE

Different antigen-presenting cells (APCs) including dendritic cells (DCs) have been found to lose their tolerogenic function in both T1D patients and NOD mice. Deregulated expression of transcriptional regulator Deaf1 was identified in pancreatic lymph nodes (PLNs) of T1D patients and NOD mice affecting expression of peripheral self-antigens required for tolerance maintenance. Both inflammation and hyperglycemia promote induction of dominant negative spliced variant of Deaf1. We initially hypothesized that defective Deaf1 function in DCs may negatively impact tolerance and contribute to progression of the disease. However, when performing mRNA sequencing on sorted CD11c+ cells from wild type and Deaf1-deficient mice, we observed an enhanced NK/cytotoxic signature in the absence of Deaf1 and confirmed that some NK cells express CD11c, prompting us to hypothesize that impaired Deaf1 function may also exacerbate cytotoxic functions and the progression of T1D. We further hypothesize that reduced function of DEAF1 in human PLNs may also affect NK cell populations and function.

METHODS

Conditional Deaf1 knockout (floxed Deaf1) mice were crossed with Cre-transgenic strains, whereby Cre is driven by CD11c or inducible CAG promoter. Lymph nodes cells and splenocytes from the different Deaf1 conditional knockout models were analyzed by multi-parameter flow cytometry and populations of interest were sorted for RNA-sequencing and qPCR. Follow-up studies include a comprehensive phenotypic analysis of NK and CD8+ T cell populations by spectral flow cytometry and multiplexed immunofluorescence by Vectra, all using fresh PLN tissue or sections from control and T1D patients obtained from nPOD.

SUMMARY OF RESULTS

Deletion of Deaf1 in CD11c+ cells resulted in a small but significant reduction of tissue resident and plasmacytoid DCs. Surprisingly, mRNA sequencing data from splenic CD11c+ cells identified a significant increase of cytotoxic/NK signature genes in absence of Deaf1, likely contributed by CD11c+ NK cells. Indeed, NK1.1+ CD49b+ NK cells from CD11c-Cre

mice lost Deaf1 expression in CD11c+ cells but not CD11c- cells. We observed an increased frequency of NKG2D+, Ly49d+ and Ly49h+ NK cells in Deaf1-deficient mice by flow cytometry. Human blood CD56+ CD11c+ and CD56+ CD11c- NK cells express DEAF1 at similar level. Studies are ongoing to assess whether Deaf1 deficiency affects NK cells (and possibly CD8+ T cells) as a whole in mouse models and whether these populations and their markers are altered in the PLNs of T1D patients where DEAF1 function is impaired. Although controversial, NK cells have been described to have a pathogenic role in T1D by infiltrating islets and contributing to beta cell killing.

CONCLUSIONS

Impaired DEAF1 function in PLNs from T1D patients may contribute to T1D disease progression by exacerbating expression of cytotoxic genes. Data to be presented will address whether subsets of CD11c+ and NK cells are affected by Deaf1 deficiency and whether impaired DEAF1 function in PLNs of T1D patients results in alterations in cytotoxic cell populations.