**SUMMARY (cutting edge) 7/11/25**

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**Source:** Excerpts from "Immunology Cell Biology - 2025 - Loaiza Naranjo - PD‐1 expressing islet‐specific CD4 T cells promote bystander tolerance.pdf"

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**Authors:** Jeniffer D Loaiza Naranjo, Vivian Zhang, Rathna Ravichandran, Anne-Sophie Bergot, Ranjeny Thomas & Emma E Hamilton-Williams

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Executive Summary

This research investigates the critical role of Programmed Death 1 (PD-1) expression on CD4+ T cells in maintaining self-tolerance and preventing autoimmunity, particularly in the context of Type 1 Diabetes (T1D). Using a CRISPR/Cas9-mediated selective knockout of PD-1 in islet antigen-specific BDC2.5 CD4+ T cells in nonobese diabetic (NOD) mice, the study demonstrates that PD-1 expression is essential for bystander suppression of autoreactive CD8+ T cells specific for different islet antigens. Loss of PD-1 in CD4+ T cells leads to their enhanced proliferation, acquisition of an inflammatory and cytotoxic phenotype, and increased infiltration into the pancreas. Crucially, this impairment in CD4+ T cell regulation subsequently promotes the proliferation and effector function of autoreactive CD8+ T cells, ultimately accelerating diabetes progression. The findings underscore PD-1's role in promoting a tolerogenic microenvironment and restraining T cell-mediated autoimmune responses, with significant implications for immunotherapy design in autoimmune diseases.

Key Findings and Themes

1. PD-1 Expression on CD4+ T Cells is Crucial for Maintaining Self-Tolerance

* **Core Hypothesis:** The study aimed to determine "Whether PD-1 expression by CD4+ T cells is necessary for induction and maintenance of bystander tolerance."
* **Mechanism of Tolerance:** T1D is characterized by "loss of T-cell tolerance to multiple islet antigens." This research focuses on "bystander suppression," where tolerance to one autoantigen induces tolerance to other T-cell reactivities.
* **PD-1's Role:** The study concludes that "PD-1 expression by antigen-specific CD4+ T-cell populations is required for bystander suppression of autoreactive CD8+ T cells of other specificities." This highlights PD-1 as a "major checkpoint inhibitor of T-cell activation and effector function."

2. Loss of PD-1 in CD4+ T Cells Leads to an Activated, Inflammatory, and Cytotoxic Phenotype

* **Enhanced Proliferation:** CRISPR/Cas9-mediated knockout of PD-1 in BDC2.5 CD4+ T cells resulted in "increased the overall proliferation of chromogranin A-specific BDC2.5 CD4+ T cells."
* **Effector-Memory Differentiation:** PD-1-deficient BDC2.5 cells rapidly "differentiated into activated (CD44hi CD62L) cells in the spleen and pLN of recipient mice." They acquired a "T-bet+ ICOS+ CD107a+ IFN-c+ effector phenotype."
* **Increased Cytotoxic Markers:** A "significant increase in the number of BDC2.5/PD-1KO cells within the pancreas, with a similar trend in endogenous CD4+ T-cell infiltration." These cells showed "Significantly higher proportions of CD107a in PD-1KO cells were observed in the pLN and pancreas and higher IFN-c overall." CD107a measures degranulation and cytotoxic activity.
* **Reduced IL-10:** "Loss of PD-1 resulted in a reduction in IL-10 expression in the spleen four days post-transfer," indicating a shift away from a regulatory profile.
* **Conclusion:** "These data suggest a role for PD-1 in preventing inflammatory and cytotoxic self-reactive CD4+ Th1 cell development."

3. PD-1-Deficient CD4+ T Cells Impair Regulation of Autoreactive CD8+ T Cells

* **Increased CD8+ T Cell Proliferation:** "Deficiency of PD-1 in transferred BDC2.5 CD4+ T cells increased the proliferation and differentiation of effector CD8+ T cells in the pLN of recipient mice."
* **Enhanced CD8+ Effector Phenotype:** IGRP-specific 8.3 CD8+ T cells, when co-transferred with PD-1KO BDC2.5 cells, "developed an ICOS+ phenotype with increased degranulation potential in the pancreas." They showed "significant upregulation of CD107a and similar trends for Granzyme B and TNF."
* **Epitope Spreading:** "A break in bystander tolerance with BDC2.5/PD-1KO cells was also evident in the endogenous CD8+ T cells, which increased granzyme B expression in the pancreas." This suggests a broader autoimmune attack.

4. Pancreatic Migration of PD-1-Deficient CD4+ T Cells is Required for CD8+ T Cell Activation

* **No APC Maturation Effect:** The study found "No differences in the expression of activation markers CD40, CD86... or PD-L1 and CD155 were observed across any APC populations." This suggests that the increased CD8+ T cell activation was not due to direct licensing of antigen-presenting cells (APCs) in the pancreatic lymph nodes (pLN) by the PD-1KO CD4+ T cells.
* **Dependence on Pancreatic Infiltration:** Treatment with FTY720, an inhibitor of lymphocyte egress from lymphoid organs, "prevented the proliferative boost of 8.3 cells in the presence of BDC2.5/PD-1KO cells." This indicates that "BDC2.5/PD-1KO cells need to migrate to the pancreas to drive inflammation and enhance the activation of the 8.3 cells." The proposed mechanism is that their infiltration and subsequent islet damage "potentially releases additional islet antigens and recruits APCs capable of presenting antigens to both CD4+ and CD8+ T cells."

5. Loss of PD-1 in CD4+ T Cells Accelerates Diabetes Progression

* **Accelerated Onset:** Mice that received BDC2.5 PD-1KO cells, with or without 8.3 cell transfer, "developed diabetes at an accelerated rate compared to the other groups."
* **Sufficient to Break Tolerance:** The study demonstrates that "loss of PD-1 in BDC2.5 CD4+ T cells is sufficient to break tolerance and provokes accelerated autoimmunity in NOD mice." Even a "small proportion of CD4+ T cells can greatly impact inflammation and autoimmunity."

6. Implications for Autoimmunity and Immunotherapy

* **Peripheral Tolerance:** The study confirms PD-1's "crucial role in sustaining peripheral tolerance."
* **Epitope Spreading:** The findings suggest that "loss of PD-1 may promote epitope spreading and drive acquisition of effector function in other T cells."
* **Immunotherapy Context:** The results have "implications for immune-related adverse events following single or combined checkpoint inhibition," a common strategy in cancer immunotherapy, which can sometimes precipitate autoimmune conditions.
* **Future Directions:** The authors believe their findings and model "could be used to understand how to enhance bystander tolerance mechanisms that will potentially increase disease protection and translation of immunotherapies to humans."

Methodology Overview

The study utilized a sophisticated approach involving:

* **Animal Model:** Nonobese diabetic (NOD) mice, a widely used model for T1D, along with NOD BDC2.5 TCR transgenic mice (for islet antigen-specific CD4+ T cells) and NOD 8.3 TCR transgenic mice (for IGRP-specific CD8+ T cells).
* **CRISPR/Cas9 Gene Editing:** Selective knockout of PD-1 in ex vivo isolated naive BDC2.5 CD4+ T cells via electroporation of sgRNA-CRISPR/Cas9, enabling the study of specific cell type effects.
* **Adoptive Transfer Experiments:** Co-transfer of PD-1KO BDC2.5 cells and 8.3 CD8+ T cells into NOD mice to observe their interactions and impact on autoimmunity.
* **Flow Cytometry:** Extensive phenotyping and functional analysis of T cell populations (proliferation, activation markers, cytokine production, cytotoxic markers) and antigen-presenting cells.
* **FTY720 Treatment:** Used to inhibit lymphocyte egress from secondary lymphoid organs to assess the requirement for T cell migration to the pancreas.
* **Diabetes Monitoring:** Regular blood glucose measurements and histology to track disease progression.

Limitations

* **Incomplete CRISPR Knockout:** The conditional knockout was not 100% efficient, and full loss of PD-1 expression required several cell divisions.
* **CRISPR Off-Target Effects:** The possibility of off-target effects from the CRISPR system could not be entirely excluded.
* **Variability:** Some discrepancies in absolute cell numbers were noted, potentially due to variations in mouse age and pre-existing insulitis.
* **FTY720 Effects:** FTY720 treatment also affected the viability and numbers of endogenous cells, which might have influenced the observed accumulation of transferred cells.

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