Exploring the Mechanisms behind Anti-PD-1/Anti-PD-L1 Checkpoint Inhibitor-Associated Autoimmune Diabetes Mellitus

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INTRODUCTION

Cancer is the second leading cause of death worldwide,[1] and thus research on improving cancer treatments is imperative. This includes examining the faults of pre-existing treatments such as chemotherapy — which can cause toxic side effects — and considering newer, safer techniques. To reduce the adverse effects of traditional treatments, a new oncotherapy has emerged: immune checkpoint inhibitors (ICI), which recruit the body's natural immune system to fight tumours.

Immune Checkpoint Inhibitors

Immune checkpoint therapy (ICT) works by overstimulating the pathways that regulate the co-signaling molecules which promote and suppress T-cell activation.[2] Programmed cell-death ligand 1 (PD-1) is an inhibitory receptor protein that is expressed on activated T cells, B cells, regulatory T cells. and natural killer cells. The binding of PD-1 to its ligands inhibits T-cell activity.[2]

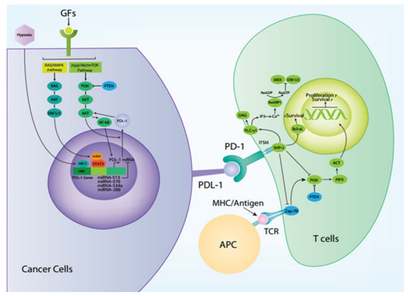

Figure 1: PD-1 antagonization of costimulatory T cell Receptor (TCR) Signaling[3]
PD-1 immunoreceptor tyrosine-based switch motif attaches to Src homology region 2. The attachment blocks the activation of TCR proximal kinases, leading to a decrease in Lck-mediated phosphorylation of TCR CD3ζ chains and zeta-chain-associated protein kinase 70. The downregulation of TCR signal transduction eventually inhibits cytokine production, T-cell proliferation and T-cell survival.

Immune checkpoint inhibitors are monoclonal antibodies (mAb) that bind to PD-1 and PD-L1, counteracting their inhibitory effects on T-cells.[3] This enhances T-cell and immune responses to induce an overwhelming immune response against tumours.[4]

HYPOTHESIS

I hypothesize that anti-PD-1/PD-L1 based immune checkpoint inhibitors lead to the development of checkpoint inhibitor-associated diabetes mellitus in individuals with human leukocyte antigen (HLA) haplotypes that are 'at risk' for traditional type 1 diabetes mellitus.

CHECKPOINT INHIBITOR ASSOCIATED DIABETES MELLITUS

Autoimmune Diseases and ICT

The immune-related adverse effects due to the enhanced immune response associated with ICI have been well-documented in the literature: examples include colitis, hepatitis, pneumonitis, and hypothyroidism.[5]

A mechanism for ICI-induced autoimmune diseases has been theorized. ICI's impact of the immune system's tolerance of tumour antigens could lead to an overproduction of autoantibodies and autoreactive T and B cells,[2] inducing hyperactivity of adaptive and innate immune cells.[4] The resulting excessive production of chemokines and cytokines result in inflammation, tissue damage, cell proliferation, and apoptosis.[4]

CIADM

There have been several reported cases of PD-1 checkpoint inhibitor associated diabetes mellitus (CIADM), and preexisting research has linked the two.[6] Since PD-1 expression is induced by proinflammatory stimuli in tumour cells and that same PD-1 is expressed in the beta cells of individuals with type 1 diabetes (T1D), ICI mediated beta cell loss may lead to CIADM.[7] Prior research has suggested that since risk for T1D can be predicted via human leukocyte antigen haplotypes (HLA), the same may be true for CIADM.[7]

RESEARCH AIMS

This project seeks to understand the mechanisms and risk factors behind checkpoint inhibitor-associated diabetes mellitus.

1. The Correlation between Anti-PD-1/PD-L1 ICI and CIADM

I aim to establish a correlation between receiving anti-PD-L1/PD-1 ICI treatment and the onset of CIADM.

2. Establishing a Relationship between PD-L1, Anti-PD-1/PD-L1 ICI and CIADM

I aim to establish a relationship between the PD-1-PD-L1 pathway and the incidence of CIADM via mouse models.

3. Identification of At-Risk, Neutral, and Protective HLA Haplotypes for CIADM

I aim to identify whether HLA haplotypes that predict traditional T1D have the same relationship with CIADM.

METHODS

1. The Correlation between Anti-PD-1/PD-L1 ICI and CIADM

I will perform a retrospective cohort study of UHN patients who have received anti-PD-1/PD-L1 treatment and have experienced onsets of hyperglycemia that requires exogenous insulin treatment by collecting the following variables.

- Age
- Race
- Pre-existing insulin deficiencies
- HLA typing
- Gender
- Time to CIADM onset
- GAD[65], IA-2 and ZnT8 diabetes autoantibodies

2. Establishing a Relationship between PD-L1, Anti-PD-1/PD-L1 ICI and CIADM

a. Generation of Cre/Lox Tissue-Specific Knockout Mice

Anti PD-L1 and anti homozygous NOR/LtJ insulitis-resistant mice and homozygous NOD/ShiLtJ mice will be loxP-flanked for the PD-L1 encoding CD274 gene that resides in chromosome 9p24. The NOD mice will be crossed with NOD/ShiLt-Tg(Ins2-cre)5Lt/LtJ and the NOR mice will be crossed with B6(Cg)-Ins1tm1.1(cre)Thor/J, both of which are pancreas specific cre transgenic mice, to create mice in which PD-L1 has been knocked out within the pancreas.

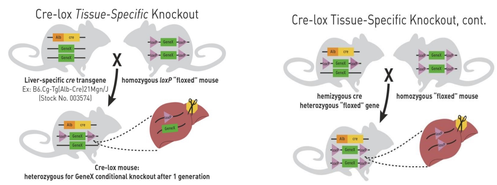

Figure 2: *Cre-Lox* Tissue Knockout Mice[8]

b. Identifying PD-1's Influence on CIADM

The resulting mice strains (4 *cre-lox* and 4 control) will be monitored over a 20 week period to determine when, if at all, the mice develop CIADM. This will address whether PD-1 modifies the course of autoimmunity in vivo, and the difference in the variable instances of T1D in NOD vs NOR mice will establish whether CIADM solely occurs in diabetes-prone environments.

C. Identifying the Influence of anti-PD-L1 mAb on CIADM

Purified anti-mouse CD279 (PD-1) mAb will be intraperitoneally introduced to the various mice strains. Should these results significantly differ from section B, it would indicate that factors unrelated to the absence of the PD-1/PD-L1 pathway contribute to CIADM.

3. Identifying At-Risk, Neutral, and Protective HLA Haplotypes

a. Determining the Accuracy of HLA Haplotype Predictions of CIADM

The four strains of mice produced in aim 2 will be backcrossed with C57B46^DRB1*04, C57B46^DRB1*03, C57B46^ DQB1*0302, C57B46^DRB1*1602, C57B46^DRB1*15:01 (DR15)-DQA1*01:02-DQB1*06:02 (DQ6), and C57B46^DR-2 mice for 20 generations while continuously selecting for the HLA alleles, until we obtain 99.99% pure NOD/NOR mice with their respective HLA alleles.

b. The Effect of HLA Haplotypes on the outcome of ICI

20 mice of each strain will be monitored and the timeline of CIADM development will be noted to identify whether the HLA haplotypes that traditionally indicate risk for T1D (DRB1*04 DRB1*03) do the same in CIADM.

Anti-mouse PD-1 mAbs will then be injected to identify whether HLA haplotypes affect the outcome of ICI mAbs on CIADM.

FURTHER DIRECTIONS

Should the outcomes of these proposed experiments affirm the hypothesis, further research must be conducted: if aim 2 indicates that the absence of the PD-1 pathway induces CIADM, clinical trials exploring the effects of varying ICI dosages on CIADM can begin. Aim 3 will have significant clinical implications — should certain HLA haplotypes impact CIADM, physicians can begin early monitoring of patients with at-risk haplotypes to mitigate the severity of CIADM. The most interesting aspect of this work is the potential to identify a new mechanism in which an autoimmune reaction (CIADM) occurs and detect potential at-risk genotypes.

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