TreatingT1D by dampening MHC-I hyperexpression in pancreatic cells through an LNP-delivered epigenetic silencer

Type 1 diabetes is caused by T-cells recognizing beta cells in the pancreas as antigenic. These T-cells bind to MHC-I "antigen-presenter" proteins expressing insulin fragments long enough to trigger an immune response. This is partly because these T-cells are 'miseducated' to recognise insulin as an antigen, and partly because beta-cells in T1D hyperexpress MHC-I. This experiment will investigate the possibility of treating this disease via epigenetic silencing in beta cells, which, being sessile, are much easier to target for treatment than the cosmopolitan T-cells. Chen's lab has demonstrated that LNPs can be highly effective at delivering RNP-Cas9 complexes to the lung; it's possible this same method can be exploited to deliver EpiReg-T silencers to pancreatic cells (Chen et al., 2024). Epigenetic silencing is generally preferable to editing where possible because it eschews the many complications of the editing's on-and-off target effects, such as chromothripsis or the possibility of inviting an anti-Cas9 immune response (which would be particularly problematic in this case). The use of a TALE-like effector as opposed to Cas9, in the style of Mao et al.'s 2025 paper, would avoid the latter possibility – not to mention the fact that TALE-like effectors were found to be dramatically more effective than dCas9 silencers in that experiment (possibly for that reason). The greater peripherality of epigenetic silencing also makes it more palatable for patients than gene therapy. To address the problem of how to treat T1D via epigenetic silencing, this proposal aims to 1. identify a gene the silencing of which will dampen, without altogether eliminating, MHC-I expression in T1D islet cells, 2. optimize Mao's EpiReg-T-carrying LNP vector to target the Islet of Langerhans, and 3. gain an understanding of whether or not autoimmunogenicity is an all-or-nothing condition or if it has safely exploitable gradations.

Experimental approaches

Aim 1: Identify a gene which, if silenced, would reduce MHC-I expression levels in beta-cells to the baseline level required for healthy immunogenicity.

This will be accomplished by ex-vivo silencing of three candidate genes in T1D pancreas explants, followed by MHC-I staining with anti-HLA-A/B/C (human MHC-I) antibodies, and quantification through flow cytometry. The MHC-I density of these cells will be compared to that of non-diabetic controls. These cells will be silenced with a TALE-like EpiReg. The candidate genes will be NLRC5 and WSHC1.

Reduced NLRC5 and WSHCI expression have both been shown to correlate with reduced MHC-I expression, and, in turn, reduced immunogenicity in cancer cells (Shukla et al., 7 and Ren et al., fig 3). This both suggests that silencing these genes will likely reduce MHC-I expression and that it is very dangerous. That being said, because it's not the only factor in regulating MHC-I expression, and because the latter is abnormally high in T1D islet cells, it is possible that these alternative mechanisms will preserve a baseline. The question of whether or not MHC-I levels are consistently high in T1D islet cells, or if they are destroyed purely from infrequent episodes of MHC hyperexpression, is significant; if the latter is the case, that would

mean that the dampening effect of a silencer would put MHC levels below the threshold for immunogenicity most of the time, causing a cancer risk.

To create the silencer for the ex-vivo part of this experiment, Mao's lab's method for building EpiReg-T will be replicated. Target sequences from these two genes will be translated to repeat-variable residue code to create two TALE repeat-arrays (one for each gene) that will be assembled using Golden Gate cloning (Mao et al., 2025, 10, "Plasmid construction"). These TALE repeat-arrays will be fused to a DNA methylation domain (e.g. DNMT3A) and a repression domain (e.g. ZIM3) (Mao et al., 2025, 2, "In vivo optimization of EpiRegs"). At this stage, an LNP vector is not necessary, so in-vivo transcription with an RNA polymerase and mRNA transfection will be used to make the explants express these effectors.

Alternative approach: In the case that silencing either of these genes pushes T1D cells below a safe baseline level of MHC-I expression, regulators downstream of NLRC5 and WSHCI will be used.

Aim 2: Optimize Mao et al.'s EpiReg-T-carrying LNP vector to target the Islet of Langerhans.

Mao's silencer targets the liver, where LNPs naturally accumulate. Jiaqi Lei and their team discovered that large LNPs (~300nm in diameter) target the mouse pancreas 3.64 times as much as the liver thanks to the comparative thinness of the connective tissue surrounding the pancreas, which allows enlarged LNPs to pass through where the liver and the spleen would not (Lei et al., 2025, fig 1e, last graph, and 1f). Lei's lab increased the natural accumulation of proteins that LNPs undergo when in contact with biological fluids by attaching arginine to the lipids, creating 'armored LNPs'. Arginine makes the surface of these LNPs more positively charged and consequently more chemically interactive. Their LNP, however, primarily delivered to stromal cells, not beta-cells. The second aim of this proposal, then, is to engineer a positively-charged LNP after the one created by Lei's lab that would be capable of delivering the mRNA for any epigenetic regulator not just to the pancreas at large but specifically to islet cells. This could be accomplished by modifying the LNP with a GLP-1 receptor agonist, as islet cells are the only cells in the pancreas that express these receptors.

Alternative approach: If the formation of a massive protein corona interferes with the function of islet-recognition receptors, or if the protein corona promotes transfection to stromal cells in addition to the islet cells even in the presence of GLP-1s agonists, one could engineer an LNP that is massive in diameter to begin with and does not need protein coronas, as well as neutrally charged – a "naked giant" that avoids stromal cells and favors islet cells.

Aim 3: Gain a better understanding of the plasticity of the MHC-I system and of autoimmunity at large.

In particular, the results of Aim 1 will be used to inform our understanding of how tolerant this mechanism is to change. The way the immune system of individual cells respond to these changes might inform whether autoimmunogenicity is primarily an issue of visibility or something else.

References

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