CELL REGENERATION

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The involvement of interleukin-22 in the expression of pancreatic beta cell regenerative *Reg* genes

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Abstract

Background: In Type 1 diabetes, the insulin-producing β-cells within the pancreatic islets of Langerhans are destroyed. We showed previously that immunotherapy with Bacillus Calmette-Guerin (BCG) or complete Freund's adjuvant (CFA) of non-obese diabetic (NOD) mice can prevent disease process and pancreatic β-cell loss. This was associated with increased islet Regenerating (*Reg*) genes expression, and elevated IL-22-producing Th17 T-cells in the pancreas.

Results: We hypothesized that IL-22 was responsible for the increased *Reg* gene expression in the pancreas. We therefore quantified the *Reg1, Reg2,* and *Reg3*δ (INGAP) mRNA expression in isolated pre-diabetic NOD islets treated with IL-22. We measured *IL-22,* and *IL-22 receptor(R)*-α mRNA expression in the pancreas and spleen of pre-diabetic and diabetic NOD mice. Our results showed: 1) *Reg1* and *Reg2* mRNA abundance to be significantly increased in IL-22-treated islets *in vitro*; 2) IL-22 mRNA expression in the pre-diabetic mouse pancreas increased with time following CFA treatment; 3) a reduced expression of *IL-22R*α following CFA treatment; 4) a down-regulation in *Reg1 and Reg2* mRNA expression in the pancreas of pre-diabetic mice injected with an IL-22 neutralizing antibody; and 5) an increased islet β-cell DNA synthesis *in vitro* in the presence of IL-22.

Conclusions: We conclude that IL-22 may contribute to the regeneration of β-cells by up-regulating Regenerating *Reg1* and *Reg2* genes in the islets.

Keywords: Adjuvant immunotherapy, Interleukin-22, Regenerating (Reg) genes, Beta-cell regeneration, Type 1 diabetes

Background

Type 1 diabetes (T1D), also known as juvenile diabetes, is an autoimmune disease characterized by the destruction of insulin-producing β-cells within the pancreatic islets of Langerhans. Type 1 diabetes is thought to be caused by a complex interaction between environmental and genetic factors that is still not fully understood [1]. This interaction causes initial damage to the pancreatic islets leading to a T-cell mediated autoimmune response that induces dysregulation and destruction of β-cells via the release of inflammatory cytokines [2]. Current T1D management includes insulin therapy and pancreas or islet cell transplantation; however, none of these procedures will ensure the complete removal of diabetic complications. Therefore, studying the endogenous regeneration of pancreatic β-cells may suggest strategies for alternative and long-lasting approaches for T1D management [3].

Endogenous β-cell regeneration is thought to occur either by whole islet neogenesis (WIN) via the differentiation of progenitor cells within the adult pancreas, or by β-cell replication (BCR) and the regeneration of new β-cells from pre-existing β-cells [3,4]. Using non-obese diabetic (NOD) or streptozotocin-injected C57/BL6 mice as a model for T1D, several groups have shown a possible role for transcription factors, growth factors, and regeneration genes in stimulating β-cell regeneration. This could result in whole islet neogenesis (WIN) and subsequent insulin production

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and diabetes reversal. Some of these candidate factors include pancreatic and duodenal homeobox 1 (PDX-1), glucagon-like peptide-1 (GLP-1), islet neogenesis-associated protein (INGAP) and Regenerating protein 1 and 2 (Reg1 and Reg2) [3,5-9]. Recently, platelet-derived growth factor (PDGF) has also been shown to stimulate β-cell regeneration via activating cyclin D1 and inducing a G1 to S transition of the β-cell cycle [10].

Previously, we have shown that a single injection of *Mycobacterium*-containing Complete Freund's Adjuvant (CFA) into NOD mice has a protective effect against T1D by down- regulating autoimmunity and restoring normoglycemia via the induction of various regulatory T (Treg) cells [7,11,12]. The role of CFA on endogenous β-cell replenishment, identified by histological analysis, however, still remains controversial and the exact mechanism is presently unknown [5,7].

Several groups have identified the Regeneration gene family (*Reg)* as being expressed during the process of WIN and β-cell regeneration in the pancreas [3,5,7,9,12]. There are seven types of *Reg* genes present in the mouse (located on chromosome six with the exception of *Reg*4) but only five have been shown to be present in the human (located on chromosome 2) [13]. The Reg proteins encoded by these genes are C-type lectins, and have been found to be also expressed in a variety of tissues such as the liver, kidney, brain, and gastrointestinal tissues [3,13]. Once secreted, these soluble proteins act in an autocrine and/or paracrine manner to exert their effects on their cognate receptors, where they may stimulate an anti-microbial, anti-inflammatory, anti-apoptotic, or regenerative response depending on the tissue type [3,7,9,13]. In the last decade, *Reg*1 and *Reg*3δ (INGAP), expressed by β-cells and acinar cells respectively, have been shown to be linked with pancreatic β-cell regeneration in the mouse by activating cyclin D1 and promoting β-cell cycle progression [9,13,14]. More recently, we have shown that *Reg2* is substantially up-regulated in the pancreatic islets, particularly in the β-cells, following adjuvant treatment in diabetic and non-diabetic NOD female mice, as well as in C57BL/6 mice treated with streptozotocin (STZ). This increased *Reg2* expression has been shown to be associated with an increase in insulin production, a partial reversal of insulitis, and an improved glucose tolerance test in STZ-treated diabetic C57BL/6 mice [5]. This led to the conclusion that β-cell regeneration via up-regulation of the *Reg2* gene may have the capacity to reverse T1D in diabetic mice immunized with CFA using a pathway that is similar to Reg1 and Reg3δ [5,7].

CFA immunization has been shown to induce CD4+ Th17 T cells to produce interleukin IL-17, IL-22, IL-10 and IFN-γ in NOD mice [15]. This finding leads to the concept that adjuvant-induced cytokines may have the potential to activate transcription factors that stimulate Reg proteins such as Reg1, Reg2, and Reg3β (PAP1) [7]. Among these cytokines, IL-22 has been of specific interest because it is released by CD4+ Th17 T cells in diseases such as hepatitis and inflammatory bowel disease, where IL-22 expression levels have been shown to promote cell regeneration and survival in hepatocytes and colonal epithelial cells. Interestingly, IL-22 has also been found recently to induce *Reg* gene expression in the pancreatic acinar cells surrounding pancreatic islets [16]. IL-22 is a member of the IL-10 family of cytokines and the gene is located on human chromosome 12. Like IL-17A and IL-17F, IL-22 is a glycoprotein, which binds to its receptor complex (composed of IL-10Rβ and IL-22Rα) as a homodimer [17,18]. The IL-22 receptor complex is highly expressed in the pancreatic α and β cells [19]. Upon receptor binding, the tyrosine kinases, JAK and Tyk1 are phosphorylated which leads to the activation of the STAT3 transcription factor and subsequent up-regulation of the *Reg* genes in the mouse and human [17,18]. However, the possible induction of the *Reg* genes via IL-22 in the pancreatic islets or NOD mouse model has not yet been examined.

This study sought to test the effects of IL-22 on the regulation of the pancreatic *Reg* genes, with a focus on *Reg*2, in diabetic and pre-diabetic NOD female mice immunized with CFA. This was accomplished in a two-step process: Firstly, by studying the direct effect of IL-22 on *Reg* gene up-regulation in the NOD mouse pancreatic islets; and secondly by examining whether CFA immunization could up-regulate *IL-22* expression in the whole pancreas. It was hypothesized that CFA immunization in pre-diabetic mice would lead to the production of IL-22 via the induction of Th17 cells, and that the resulting IL-22 cytokine would activate a JAK-STAT3 signal transduction pathway following binding to its receptor complex on pancreatic β-cells. IL-22 signaling would then result in the up-regulation of *Reg* gene expression that may be linked to β-cell regeneration and the reversal of hyperglycemia in T1D. This, however, requires inhibition of autoimmunity to prevent the reversal of disease. Immunotherapies with mycobacterial adjuvants such as BCG vaccination have the potential to achieve both these objectives [20].

Results

Recombinant IL-22 up-regulates Reg2 and Reg1 mRNA expression in the pancreatic islets

To establish whether IL-22 induces *Reg* gene expression, 6-week-old pre-diabetic NOD mouse islets were isolated and incubated with recombinant IL-22 at 10 and 50 ng/mL concentrations or supernatants from Th17 polarized splenocytes containing IL-17 and IL-22 [15] (Figure 1). Quantitative RT-PCR was performed on total extracted