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IL-4R is expressed on alpha and beta cells of human pancreata

Sakthi Rajendran, **Madeleine Graef**, **Tiffany Chu**, **Matthias von Herrath***

La Jolla Institute for Immunology, La Jolla, CA, United States of America

1. Introduction

Type 1 diabetes (T1D) is widely regarded as a Th1-mediated disease, where beta cell killing is facilitated by IFN-γ driven activation of CD8+ T cells and macrophages [1,2]. T1D is often accompanied by reduction in Th2 responses, for instance, stimulated PBMC or T cells from patients with recent onset T1D exhibit reduced capability of secreting IL-4 [3]. IL-4, being a major driver of Th2 polarization, was extensively studied in the context of preventing T1D, due to its antagonizing effects on Th1 responses. Several studies in the NOD mice in the 1990s reported that i) expression of IL-4 in the beta cells of NOD mice model prevents insulitis and incidence of T1D [4], and ii) treatment of NOD with recombinant IL-4 prevents diabetes and reverses T cell proliferative unresponsiveness before the onset of diabetes [5,6]. IL-4-mediated protection from T1D is reported to be driven by activation of non-pathogenic T cell clones [7] and induction of regulatory function of Th2 cells [8]. In addition, exposure of isolated human islet cultures to IL-4 has been shown to protect islets from pro-inflammatory cytokine induced cell death, mainly though activation of the STAT6 pathway [9,10]. It is not clearly understood whether the protective effect of IL-4 in preventing T1D is solely due to localized immunosuppression in T cells or in part due to IL-4R signaling in beta cells. There are also contradicting evidences regarding the expression of IL-4R in human pancreas. IL-4R expression has been reported to be expressed in human islets at both protein [6] and mRNA [11] levels. Levels of IL-4R mRNA in human islets were further enhanced upon infection with Coxsackie virus B (CVB) or treatment with pro-inflammatory cytokine cocktail [12]. Contrarily, data from human protein atlas suggests low or no expression of IL-4R in islets of human pancreas [13]. This area has been relatively unexplored in nearly a decade, warranting subsequent studies. Hence, we reinvestigated the expression of IL-4R in human pancreas and traced its cellular source using multiplex florescence imaging.

2. Methods

Formalin Fixed Paraffin Embedded (FFPE) sections of pancreas from a non-diabetic donor (6373, age 15.7 years, retired case) were obtained from nPOD (Network for Pancreatic Organ donors with diabetes). After antigen demasking with citrate buffer (pH 6) at 95 °C for 20 min, the slide was stained with mouse anti-IL-4R (MAB230, R&D systems at 1:25), anti-Insulin-AF488 (53–9769-82, ebioscience at 1:400) and anti-Glucagon (Abcam, ab10988,

*Corresponding author at: La Jolla Institute for Immunology, 9420 Athena Circle, La Jolla 92037, United States of America. matthias@lji.org (M. von Herrath).

conjugated in-house with AF555 at 1:100). Section of human tonsil was used as a positive control. The staining pattern of IL-4R in pancreas was confirmed using another antibody for anti-IL-4Rα (Thermo PA5–36522). Whole tissue imaging was performed using Zeiss axioscan Z1. Ten islets were randomly selected across the whole tissue and cropped for further analysis. Original images of individual channels were exported from Zen. Image analysis was performed using ImagePro Premier. Islet areas were quantified based on insulin and glucagon staining. Quantification of positive area was performed using smart segmentation function in ImagePro Premier. Overlap area analyses of insulin-IL-4R and glucagon-IL-4R were performed by parent-child analysis.

3. Results

IL-4R consists of two subunits, IL-4Rα and γc, which upon activation, signals through the JAK-STAT pathway. In our study, we stained for IL-4Rα, as the other subunit γc is also shared by multiple receptors including IL-2R and IL-7R. We found that IL-4Rα was strongly expressed in all the islets of the non-diabetic donor (nPOD 6373), Fig. 1A. IL-4R expression was observed in 45.3 ± 11.22% of the total islet area (Fig. 1B). IL-4R was also expressed at very low levels in parts of the exocrine tissue (Fig. 1A). Expression of IL-4R was particularly stronger in the islets compared to the exocrine tissue, which supports the previous findings using anti-sera against IL-4R in human pancreata [6]. We further examined the localization of IL-4R expression within the islets. Both beta and alpha cells in the islets widely expressed IL-4R. Beta cells contributed to 53.7 ± 24.8% of the total IL-4R-positive area (Fig. 1C), while alpha cells contributed to 31.0 ± 16.4% of the total IL-4R-positive area (Fig. 1D). In particular, all alpha cells (99.7 ± 0.9%) and most beta cells (64.3 ± 20.2%) expressed IL-4R (Fig. 1F, E). Our findings confirm that IL-4R is expressed in the islets of the human pancreas. Subsequent mechanistic studies in human islets are required to evaluate if signaling through the IL-4R can protect beta cells from immune attack in T1D.

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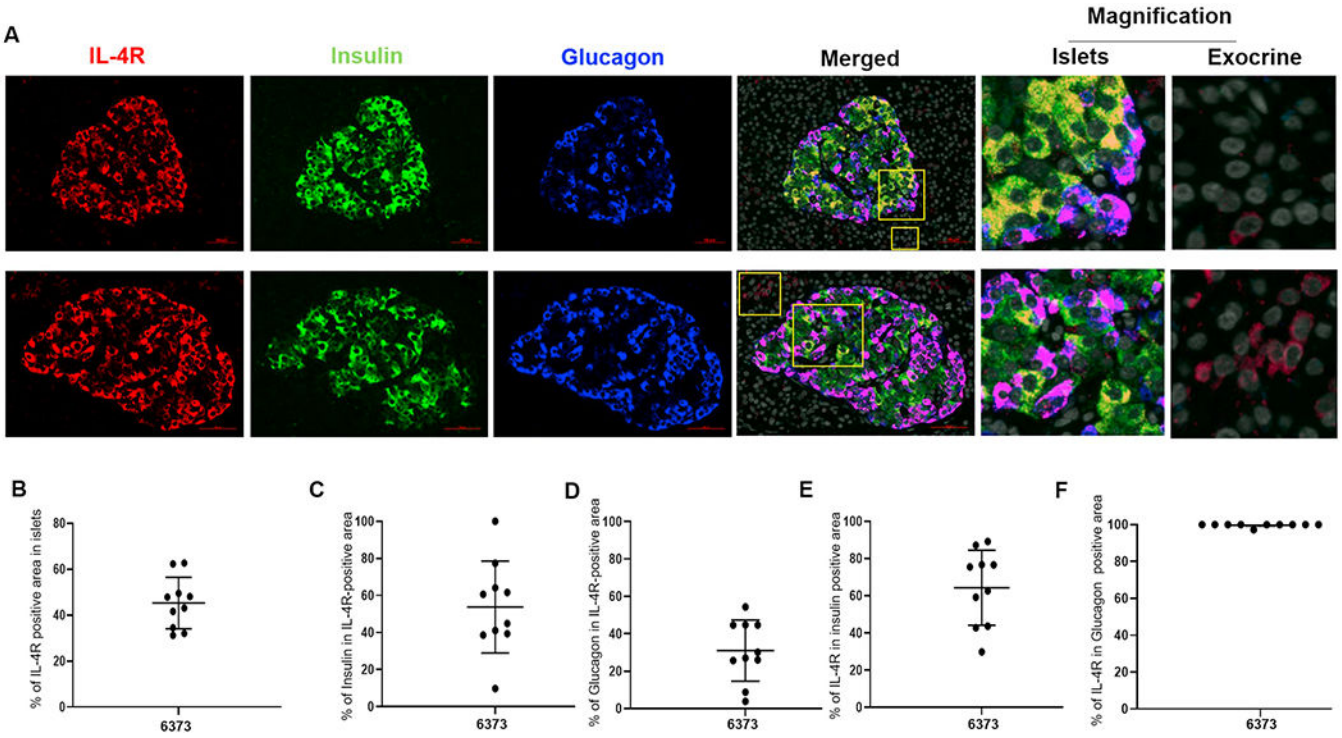

Fig. 1.

IL-4Rα is expressed on the islets of non-diabetic human pancreata: Representative images of islets from a non-diabetic donor (6373) tissue stained for IL-4Rα (red), Insulin (green), Glucagon (blue) and Hoechst (white) (A). Ratio of IL-4R-positive area to area of the islet (B), Proportion of beta cells in IL-4R-positive area (C), Proportion of alpha cells in IL-4R-positive area (D), Percentage of beta cells expressing IL-4R (E) and Percentage of alpha cells expressing IL-4R (F). Represented are Mean ± SD values of individual islets. Images were acquired using Axioscan Z1 at 20×. Scale bar – 50 μM. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)