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IDENTIFICATION OF ENTEROVIRUS RECEPTOR DECAY ACCELERATING FACTOR (CD55) IN HUMAN PANCREATIC ISLET CELLS

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Type 1 diabetes mellitus results from the selective autoimmune destruction of pancreatic islet β-cells, triggered and/or accelerated by environmental agents. One of the environmental risk factors is represented by enteroviral infection. Coxsackievirus B4 has been isolated from patients with acute onset type 1 diabetes, and some of these isolates have been reported to cause diabetes in mice. In cultured human islet cells, several enterovirus strains can replicate, suppress insulin release and, in a few cases, cause β-cell destruction. As for the islet cell enterovirus tropism, the potential mechanisms may include the expression of specific enterovirus receptors such as coxsackie-adenovirus receptor (CAR) and decay-accelerating factor (DAF-CD55); however, no data on CAR or DAF expression in human islet cell subsets is at present available. In the present study, we aimed at determining the expression of DAF in human pancreas, as a potential way of entry of enteroviruses into islet cells. To this end, by immunohistechemistry and confocal microscopy analysis we stained pancreatic sections from 6 normal control and from 2 type 1 diabetic pancreata utilizing an anti-DAF specific antibody. Signs of enteroviral infection were present in the 2 diabetic pancreata. A specific immunostaining for DAF was observed mainly in pancreatic islets, with few scattered exocrine and ductal cells resulting positive as well. Of note, a particularly strong posiotivity was observed in type 1 diabetic islets where an enteroviral infection was demonstrated. Of note, the cellular distribution of enterovirus positivity mirrored that of DAF staoining. In conclusion, these data provide the first evidence of the expression, by pancreatic islet cells, of a specific enteroviral receptor which may represent the molecular basis of enterovirus tropism in the pancreas.

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DECREASED REGULATORY $CD4^+CD25^+$ T_{REG} CELLS IN NEWLY DIAGNOSED TYPE 1 DIABETES

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Type 1 diabetes (T1DM) results from chronic autoimmune destruction of β–cells in genetically predisposed individuals.The persistence of autoreactive T cells directed towards β–cells suggests that both central and peripheral tolerance mechanisms are compromised in T1DM patients. Nowadays, $CD4^+CD25^+T$ cells (T_{reg}) represent a distinct lineage of "professional" suppressor cells thought to act through direct contact with responder cells without release of cytokines. The elimination of this $CD4^+T$ cell subset by Ab's to CD25 potently induces autoimmunity in animal models. Our aim was to analyse Treg percentages in 18 newly diagnosed Type 1 diabetic patients with recent onset of disease (within 2-8 weeks from their diagnosis) in comparison to control subjects. All patients were positive for at least one of the β-cell autoantibodies analysed (ICA, GAD65, IA-2 and IAA). Fresh PBMCs were isolated on Ficoll gradients and analysed by flow cytometry using specific monoclonal antibodies. Cytokine production (IFN-γ and IL4) was assessed by flow cytometry on PMA+Ionomycin+brefeldin activated (for 4 hours) $CD4^+T$ cells. A significant deficiency of T_{reg} cells was regularly seen in T1DM patients in comparison to normal subjects (3.6 ± 0.25 vs 6.1 ± 0.2 %, $p < 0.001$), while no difference was found in $CD8^+CD25^+$ cells (2.1 ± 0.5 vs 1.9 ± 0.6 %, p NS). An increase in $CD4^+IL4^+$ cells (Th2) (19.5 ± 2.7 vs 12 ± 3%, $p < 0.01$) and a dramatic decrease in $CD4^+IFN\gamma^+$ cells (Th1) was found in T1DM patients in comparison to control cells (20 ± 4.8 vs 55 ± 3.2%, $p < 0.001$). Our results show that T_{reg} cells are deficient in T1DM patients at the onset of the disease. In addition, $CD4^+$ cells from T1DM patients are characterized by a profound defect in Th1 cytokines, confirming that there is an underlying immunoregulatory T cell defect in the disease.

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BETA-CELL DIFFERENTIATION AND MATURATION IN HUMAN ISLETS AND THE EFFECT OF TYPE 2 AND TYPE 1 DIABETES

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Several transcription factors (TrF) are involved in the differentiation and maturation of pancreatic beta-cells. No information is currently available as for the expression of these TrF in isolated, adult human islets (Isl), the possible changes in the presence of Type 2 (T2D) or Type 1 (T1D) diabetes, and the relation with Isl physiopathology. Purified Isl were prepared from the pancreas of 3 control multiorgan donors (Ctrl, age: 38±18 yrs, M/F: 2/1, BMI: 25.1±0.9 kg/m2), 3 donors with T2D, (age: 68±3 yrs, M/F: 1/2, BMI: 28.3±0.9, duration of diabetes: 3.1±1.5 yrs), and 2 donors with T1D (age: 19±7 yrs; M/F: 1/1, BMI: 23.3±6.1 kg/m2, duration of diabetes: 1.0±0.0 yrs). Isl mRNA expression of various TrF was then measured by semiquantitative RT-PCR after 3-day euglycemic culture, and the results expressed as the ratio over beta-actin. PDX-1 expression was 0.26±0.05 in Ctrl, and was significantly ($p<0.01$) higher in T2D (0.69±0.03) and T1D (0.49±0.01) Isl. The expression of Nkx6.1 was 0.62±0.08 in Ctrl, and resulted higher ($p<0.05$) in T2D (0.81±0.02) and lower ($p<0.01$) in T1D (0.20±0.01). Nkx2.2 and PAX-6 were similarly expressed in Ctrl (respectively 0.76±0.02 and 0.51±0.06) and T2D (respectively 0.71±0.02 and 0.60±0.01), but significantly less expressed in T1D (respectively 0.32±0.03 and 0.05±0.00, both $p<0.01$). The amount of apoptotic cells (expressed in arbitrary units of optical density, OD) was higher in T2D (1.7±0.3, $p<0.05$) and T1D (1.8±0.6) than in Ctrl (0.9±0.1), and insulin content (μU/islet) was 118±14 in Ctrl, 77±26 in T2D ($p<0.05$ vs Ctrl) and 26±8 in T1D ($p<0.05$ vs Ctrl and T2D). In conclusion: 1) isolated, adult human pancreatic Isl express a number of TrF involved in beta-cell differentiation and maturation; 2) T2D have increased/normal and T1D have decreased (with the exception of PDX-1) expression of these TrF; 3) in the presence of similar apoptotic rate, the different expression of TrF may contribute to the different insulin mass in T2D and T1D Isl.

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25 (OH) VITAMIN D3, 1,25(OH)2 VITAMIN D3 AND RESIDUAL BETA-CELL FUNCTION IN PATIENTS WITH TYPE 1 DIABETES

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Background: As shown by epidemiological retrospective studies and a recent Finnish prospective study, supplementation with vit D3 at birth protects individuals from type 1 diabetes later in life. Vit D3, and especially its activated form 1,25-dihydroxyvitamin D3, have an effect on the immune response switching from TH1 to a TH2 cell response.
Patients and methods: Recent onset patients with type 1 diabetes (n=61) were investigated (32 F,29 M, mean age 14.6 years). As control group 57 age and sex matched normal subjects (24 F,33 M, mean age 16.5 years) were included. Metabolic parameters including residual C-peptide function were evaluated. Plasma levels of both forms of Vitamin D3 were measured by radioimmunoassay.
Results: Mean levels of 25 (OH) vit D3 were 36.5 ng/ml±28.6 and C-peptide was 1.0 ng/ml±0.5 with no correlation with residual beta cell function (r=0,2). Mean levels of 1,25 (OH)2 vit D3 were 23.9 pg/ml±22.6 with also no correlation with residual beta cell function (r= 0,12). Mean levels of both forms of vit D3 were significantly lower in diabetic patients compared to controls ($p<0.01$ and $p<0.03$, respectively). There was no correlation between 1,25 (OH) 2 vit D3 levels and the status of metabolic control at diagnosis, age, sex and seasonality of disease diagnosis.
Conclusion: Plasma levels of both forms of Vit D3 are reduced in type 1 diabetes at diagnosis.This finding suggests that vit D3 may be an important pathogenic factor in this disease and its supplementation may be considered at disease onset.