its complications. Towards this end, a cross-sectional study was conducted to investigate the association between two promoter polymorphisms in the TNF-α gene (–308 G>A, rs1800629, and –238 G>A, rs361525) in an Asian Indian diabetic subjects with neuropathic foot ulceration. The study comprised a total of 582 participants. PCR-RFLP based genotyping was carried out on 103 NGT (44M:59F), 116 T2DM (61M:55F) without complications and 108 T2DM with diabetic foot ulcer (DFU) (60M:48F) for -308 G/A polymorphism and 91 NGT (39M:52F), 77 T2DM without complications (40M:37F) and 87 DFU (50M:37F) subjects were screened for -238 G/A polymorphism. The serum levels of TNF-α was measured using ELISA kit. PVD subjects were excluded from the study. No significant differences regarding genotype distribution or allelic frequencies were found between patients and control subjects for the –238 G>A polymorphism [odds ratio =0.81; CI= 0.47-1.39; p=0.533). On the other hand, the allelic frequency of "A" in the –308 polymorphism was 58% in NGT, 49% in T2DM and 64% in DFU and showed a significant association with DFU (P < 0.001). The unadjusted odds ratio for DFU for the A/A genotype was 1.98; [CI= 1.09-3.59; p=0.03], when compared with NGT and the odds ratio of "A"allele was 1.29; [CI= 0.87-1.91; p=0.23]. The serum levels of TNF-α were also found to be significantly associated with DFU subjects. Hence, our data suggest that homozygous mutant "AA" allele of TNF-α –308 is likely to be a major risk factor for developing neuropathic foot ulcer in Asian Indian diabetic subjects.

IMMUNOLOGY

Immunology/ Transplantation PUBLISHED ONLY

2823-PO

Naturally Occurring IL-10+ Regulatory B Cells Prevent the Onset of Autoimmune Diabetes

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A central role for B-cells in promoting pathogenesis of type 1 diabetes (T1D) has been established; however new evidence suggests a more complex role for B-cells as regulators of autoimmune responses. Here, we characterized the islet-harbored B-cells in female NOD mice that are naturally protected from hyperglycemia. Long-term normoglycemic (Lnglc) NOD mice showed less organized islets B-cell infiltration, while the analysis of immunoglobulin heavy chain variable region (IgV_H) sequences revealed affinity maturation, clonal expansion and intraclonal isotype switch compared to hyperglycemic (Hglc) NOD mice. Interestingly, Lnglc mice comprised significantly higher levels of islet-harbored IL-10$^+$ B-cells compared to Hglc mice. The adoptive transfer of B-cells from Hglc NOD mice promotes hyperglycemia in B-cells depleted Nglc hCD20Tg NOD mice. IL-10$^+$B-cells inhibit T cell-mediated autoimmune responses *ex vivo* and diabetes transfer in NOD.Scid mice *in vivo*. Likewise, healthy individuals and relatives of patients with T1D that have detectable levels of autoantibodies in their serum, but without overt T1D, showed significantly higher levels of IL-10$^+$ B-cells in their peripheral blood compared to T1D patients, paralleling our findings in the NOD mouse model. A pool of naturally occurring highly affinity maturated, islet-harbored regulatory B-cells may protect from autoimmune diabetes.

2824-PO

WITHDRAWN

2825-PO

Association of IL-6 -174 G>C Polymorphism With Diabetes Mellitus in the Romanian Population

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Activation of the immune system and the immune mediators are related to diabetogenesis. The interactions between cytokine IL-6 -174 G>C promoter polymorphism and other factors, such as viral infections, may describe better its association with DM.

The aim of this study was to test the association of IL-6 -174 G>C polymorphism and Torque teno viruses (TTVs: TTV, TTMDV, TTMV) infection with DM in the Romanian population.

Biological samples and clinical data were collected from 400 unrelated Romanian subjects after obtaining informed consent: T1DM (n=100) and T2DM (n=100) patients and matched healthy subjects (n=200).

IL-6 -174 G>C polymorphism genotyping and TTVs detection were assessed using PCR-based methods.

The distribution of IL-6 -174 G>C genotypes was in accordance with Hardy-Weinberg equilibrium. The -174C allele had a higher frequency in patients diagnosed with T1DM in the first 10 years of life than in those with later onset (OR=2.43, 95% CI: 1.033 -5.698, p=0.04). TTVs infection was more prevalent in T1DM patients carriers of C allele than in controls (p=0.03, OR=1.9).

The prevalence of TTVs in our study was 66.5%. There was no significant difference of TTVs distribution in patients compared to controls (p=0.19). The result remained insignificant when subjects were stratified by age, gender, or BMI. Healthy males infected with TTMDV had higher BMI (p= 0.0084) and triglycerides levels (p=0.0496) compared with healthy males without TTMDV. Higher cholesterol levels were found in T2DM male patients infected with TTV (p=0.025), TTMDV (p=0.0024) or TTV+TTMDV (p= 0.0021) compared with controls.

The presence of -174C allele and infection with TTVs were more common in T1DM patients than in healthy controls. This allele has a modest association with the early onset of T1DM. The polymorphism was not associated with T2DM in our study.

Supported by: POSDRU (89/1.5/S/64109)

2826-PO

Factors and Mechanisms Controlling the Generation of Tregs in the Thymus

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Foxp3+ CD4 + regulatory T cells (Tregs) play an important role in the progression of type-1 diabetes in both human patients and mouse models. The great majority of Tregs are made in the thymus; the mechanisms involved in their generation are currently under intense investigation. This project aims to determine what factors control the differentiation of Tregs in the thymus, and whether these elements are environmentally or genetically modulated in disease states, in particular type-1 diabetes.

The Treg niche represents a thymic microenvironment composed of limited amounts of factors for which differentiating thymocytes compete in order to permit generation of Treg cells. The limited availability of such factors may control the development of naturally diverse repertoire of Tregs. The nature of these factors could be diverse, being either T cell receptor (TCR)-dependent (e.g., TCR itself, antigen) or TCR-independent (e.g., cytokines, costimulatory molecules). Different groups have previously demonstrated that TCR specificity is an essential factor of Treg niches using TCR transgenic mice expressing natural Treg TCRs. Interestingly, others recently challenged this concept using a wild type (wt) polyclonal system. The current study aims to investigate the underlying mechanisms of this unexpected observation that could have implications for our understanding of Treg cell differentiation, which could lead to new information on factors important for Treg generation that could have therapeutic utility.

For author disclosure information, see page 829.

TRANSPLANTATION

2827-PO

An Experimental Study on Treatment of Diabetic Rats through Transplantation of Allogeneic Bone Marrow Mesenchymal Stem Cells or Insulin-Producing Cells

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To observe the effectiveness of allogeneic bone marrow mesenchymal stem cells (BMSCs) and insulin-producing cells (IPCs) transplantation in diabetic rats. BMSCs were isolated from allogeneic bone marrow of SD rat by adhesive screening method and cultured in vitro and differentiate BMSCs into insulin-producing cells. The second passage of BMSC and IPCs was transfected with Ad-GFP. IPCs or BMSCs were transplanted into allogeneic STZ diabetic rats through vena caudalis. After the 2 weeks, the levels of plasma glucose and glycosylated serum protein(GSP) were determined. After 2 months, the pathologic changes of the pancreas, testicle and the expression of GFP labled cells in the pancreas were observed. The result showed that the levels of plasma glucose and GSP were lower slightly in IPCs group(IPCG) and BMSCs group (BMSCG) than in untreated group (UTG)(P 0.05). The numbers of beta-cells in IPCG and BMSCG increased and the insulin reaction showed positive and strong positive results than in UTG. There were more various grades of spermatocytes in the testis in IPCG and BMSCG than in UTG. The cells of GFP were observed in the excrine tissues of pancreas in BMSCG , but not observed in the islet and IPCG.

It was concluded that BMSCs or IPCs transplantation could depress the levels of plasma glucose and GSP in diabetic rats and might promote endogenous regeneration of islets, reduced pathological changes in diabetic testicular.

Levels of plasma glucose and GSP in each group of rats (means±SD)

	n	plasma glucose (mmol/L)	GSP (mmol/L)
NCG	6	6.22±0.62	91.1±7.9
UTG	7	20.76±2.95	139.1±12.3
IPCG	7	21.50±1.49	131.4±20.9
BMSCG	9	16.74±7.12	132.3±27.2

2828-PO

WITHDRAWN

2829-PO

Diabetes Mellitus (DM) and Impaired Fasting Glycemia (IFG) in Pre-Liver Transplant Patients (PLTP)

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Glucose metabolism alterations have heavy negative impact on liver transplant patients. However, little and inconsistent information is available on the prevalence and characteristics of DM and IFG in pre-liver transplant patients. We studied 178 PLTP patients [(age: 53±9 yrs, M/F: 131/47, BMI: 24.8±3.0 Kg/m^2, family history of diabetes: 49 (39%), fasting plasma glucose, FPG: 107±38 mg/dl, HbA1c: 34±12 mmol/mol)] who entered the waiting list for orthotopic liver transplantation in our institution. Causes of liver disease were HCV-related (HCV+) in 90 subjects (51%). Based on clinical history and FPG, 40 patients (22%) resulted to be affected by DM (age: 57±7 yrs, M/F: 32/8, BMI: 25.9±3.1 Kg/m^2), 44 patients (25%) had IFG (age: 54±7 yrs, M/F: 35/9, BMI: 24.6±2.8 Kg/m^2), 94 patients (53%) were non-diabetic (ND) (age: 51±10 yrs, M/F: 64/30, BMI: 24.6±3.0 Kg/m^2). The respective FPG and HbA1c values were: 148±62 mg/dl and 49±15 mmol/mol, 107±6 mg/dl and 32±6 mmol/mol, 90±8 mg/dl and 28±6 mmol/mol. DM subjects were older ($p<0.01$) and had a 2-fold higher rate of family history of diabetes ($p<0.01$) than ND. In this series, HCV+ was not associated with higher DM or IFG rate. In the DM group, 22% of patients were on diet alone, 22% on oral agents and 56% on insulin.

In conclusion, this study provides informations on the prevalence and characteristics of DM and IFG in patients undergoing liver transplantation; the observation that 22% of patients were on oral agents despite liver failure should prompt to develop guidelines for the therapy of diabetes in these subjects.

2830-PO

WITHDRAWN

2831-PO

Study of Glucose Metabolism One Year after Liver Transplantation

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Introduction: End-stage liver disease is associated with severe alterations in glucose metabolism. Insulin resistance and impaired glucose tolerance (IGF) are frequent. The objective was to study if these alterations improve with liver transplantation.

Patients and Methods: Prospective study with 64 patients (82.8% men) with end-stage liver disease undergoing liver transplantation from March 2010 to February 2011. Oral glucose tolerance test (OGTT) was performed before and 12 months after transplantation. The pre transplant prevalence of diabetes was diagnosed either by fasting glucose (FG) or by OGTT. Results are presented as mean (SD).

Results: Mean age was 54.3 (8.1) years and BMI 26.2 (5.8) kg/m2. 23 patients (26.4%) had overt diabetes diagnosed by FG. 79.1% of patients who underwent OGTT (all of them with normal FG) were diagnosed of diabetes, and 9.8% of IGT. 30.4% of patients who had diabetes diagnosed by FG, didn´t meet criteria for diabetes after transplantation. The insulin requirements in patients who continued to have diabetes after transplantation decreased from 0.48 IU/kg/day to 0.17 IU/kg/day ($p < 0.05$). 6 patients (9.4%) of the remaining 64 patients without overt diabetes before transplantation had diabetes diagnosed by FG after transplantation, 4 (6.3%) refused to undergo OGTT and 7 (10.9%) died. Among the patients who underwent OGTT after transplantation, 26.4% met criteria for diabetes and 30.3% for IGF. Altogether, 49% of patients with diabetes before transplantation didn´t meet criteria for diabetes 12 months after transplant (confirmed by FG and OGTT). The choice of immunosuppressive therapy was not associated with persistence of diabetes after transplant.

Conclusions: Prevalence of diabetes in patients with end-stage liver disease is high. After liver transplantation impaired glucose metabolism improve. Further studies are needed to explain the mechanisms involved.

2832-PO

Effects of Transplantation of Mesenchymal Stem Cells in High-Fat Diet-Induced Type 2 Diabetes Mellitus

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Mesenchymal stem cells (MSC) are immunomodulatory and hypoimmunogenic. These characteristics make MSC transplantation an attractive strategy to treat DM. The aim of this study is to investigate the effects of systemic MSC transplantation on glycemia regulation and pancreatic morphometric analysis of high fat diet-induced diabetes in *Swiss* mice. High fat diet-induced diabetic mice received one single or four multiple intraperitoneal injections of rat bone marrow-derived MSCs ($5\text{-}8x10^6$) (MSCD group). Control high fat diet-induced diabetic mice received only PBS injections (D group) and control non-diabetic (C group) mice did not receive injections. Fasting and nonfasting glycemia were determined weekly and glucose (GTT) and insulin (ITT) tolerance tests were performed at one, two, three and four months after MSC transplantation. Four months after MSC injection, animals were killed and pancreas was collected for histological analyses. Four months after 1 single injection of MSC, 67% of MSCD animals were considered responders (fasting glycemia <180mg/dl) and 37% were considered non-responders. Four months after 4 multiple MSC injections, 70% of MSCD animals were considered responders and 30% were considered non-responders. Pre- and post- transplant fasting glycemia of responders were significantly different. After 4 injections, fasting glycemia and glycemia response to ITT of responder MSCD animals were significantly lower compared to diabetic animals. After 4 injections, the total islet area of the responder MSCD animals was significantly bigger than the islet area of both diabetic and control animals. Beta cell volume was not different among the groups. However, alpha cell volume was significantly smaller in responder animals than in control animals. In conclusion, the results demonstrate that MSC transplantation via intraperitoneal injection restored glucose homeostasis and induced pancreatic morphometric changes in high fat diet-induced diabetes in mice.

Supported by: FAPESP; CNPq

INSULIN ACTION—ADIPOCYTE BIOLOGY

2833-PO

Effects of Insulin Glargine on Proliferation, Differentiation and Adipose Function of Human Primary Preadipocytes from Different Fat Depots

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Insulin glargine is a long-acting human insulin analogue which is widely used as basal insulin to treat diabetes, but its role on proliferation, differentiation of human primary preadipocytes and adipose function still remain unclear. In this study,we cultured primary preadipocytes from human subcutaneous (*sc.*) and omental (*om.*) adipose tissue; compare their morphological and differentiation differences. Then two kinds of preadipocytes were induced to differentiation with different dose of insulin glargine (20nM, 200nM, 500nM,1000nM and 1500nM). We observed the effects of insulin dose on adipogenic genes expression, adipokines secretion and lipolysis.Our results showed that both cells can be successfully cultured from adipose tissue and amplificated *in vitro*.

Human *sc.* preadipocytes is more slender, and proliferates more quickly, while *om.* preadipocytes were polygonal, and easier to aging. MTT results showed that insulin glargine could inhibit *om.* preadipocytes proliferation after 72h incubation, and this effect is dose-dependent, but it had no effect on the proliferation of *sc.*preadipocytes, whether in low dose or high dose. We also found that insulin at 500nM is a suitable concentration to induce differentiation. RT-PCR analysis showed that adipogenic genes such as *PPARγ, C/EBPα* had the highest expression and preadipocytes gene *Pref-1* had the lowest expression at this concentration, ELISA results showed that this concentration had the strongest adipokines (leptin, adiponectin, RBP4 and TNF-α) secretion function. Too higher insulin concentration (1000nM and 1500nM) will induce lipolysis, and lower insulin concentration (200nM) will lead to incomplete differentiation. In conclusion, insulin glargine could inhibit *om.* preadipocytes proliferation, but it had no effect on the proliferation of *sc.*preadipocytes, insulin concentration at 500 nm is a suitable concentration to induce differentiation.

Supported by: NSFC (81070661)

INSULIN ACTION—CELLULAR AND MOLECULAR METABOLISM

2834-PO

Metabolic Effects of Insulin in Human Skeletal Muscle Myotubes With Reference to Phosphatidic Acid: An Activator of mTOR

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Insulin increases expression or activates numbers of lipogenic enzymes involved into de novo lipogenesis (acetyl-CoA carboxylase, fatty acid synthase, GPAT). Phosphatidic acid, the intermediate metabolite of de novo lipogenesis is a signaling lipid that was verified as an activator of mechanistic target of rapamycin (mTOR). Currently, mTOR is emerging as a central metabolic regulator involved in nutritional control, insulin resistance, cell growth, cell proliferation and aging. The acute effects of insulin on the content of molecular species of phosphatidic acid, its precursors- long-chain acyl-CoAs and lysophosphatidic acids, and also a terminal product of de novo lipogenesis- cardiolipin were analyzed in human myotubes obtained from vastus lateralis of athlete volunteers (n=2). Myotubes were serum-starved for 4 hs and then insulin stimulated (2.0-60 nM) for 40 min in the presence of 5 mM glucose. NAD+ was measured as a marker of myotubes content.

In the basal state, the myotubes contain 16±2 pmol of long chain acyl-CoAs, 1.5±0.5 pmol of lysophosphatidic acids, 70±10 pmol of phosphatidic acids, and 300±50 ng of cardiolipin per 1 nmol of NAD^+. The major molecular species for all the metabolites are palmitoleate, palmitate, oleate and stearate containing species. Insulin induces relative increase in palmitoyl-CoA at concentration as low as 2 nM that indicates the activation of malonyl-CoA/fatty acid synthase axis. At higher concentrations (5-40 nM) insulin induces ~30% increase in the content of both lysophosphatidic acids and phosphatidic acids. There is no significant increase in cardiolipin.

In summary, the activation of malonyl-CoA/fatty acid synthase axis is an early event in the effects of insulin in human skeletal muscle myotubes. The accumulation of phosphatidic acids follows this event. We hypothesize that glucose entrance into myotubes can play role in insulin-induced increase of the phosphatidic acid in myotubes.

2835-PO

WITHDRAWN

For author disclosure information, see page 829.

ADA-Funded Research