Table 1. Minor allele distribution and association with T2DM

Gene/ SNPs	Alleles M/m	M/M	M/m	m/m	*P value	**P value
TCF7L2 rs11196218	G/A	11.03±2.30	11.53±2.72	11.13±2.42	0.1762	0.0913
TCF7L2 rs7903146	C/T	11.19±2.44	11.46±2.90	12.02±0.00	0.7859	0.5107
SLC30A8 rs13266634	C/T	10.90±2.56	11.05±2.25	12.13±2.73	0.0639	0.0937
PCSK1 rs3811951	A/G	11.25±2.45	11.12±2.55	11.46±2.45	0.7425	0.8094
PCSK2 rs2021785	G/A	11.22±2.44	11.27±2.54	11.11±2.64	0.9479	0.9522

Conclusion: Our results provide evidence that the association of PCSK2 and T2DM was also existed in Han Chinese population in Chongqing. We were underpowered to detect the association between other SNPs and T2DM or proinsulin conversion.

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IMMUNOLOGY

2533-PO

A Mathematical Model To Study the Autoimmune Progression towards Type 1 Diabetes

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Islets of Langerhans are small endocrine organs characterized by an complex anatomy including a core of insulin producing beta-cells, tightly interconnected by gap junctions and a mantle of other endocrine cells. The integrity of the interactions and the 3D architecture among beta-cells is critical for proper biosynthesis, storage and the release of insulin. The aim of this study was to evaluate the effect on beta-cells signalling of progressive lymphocytic islet cell infiltration (insulitis), by modelling the disruption of pancreatic islet anatomy as a consequence of insulitis in terms of apoptotic events of beta-cells and altered glucose concentration. We numerically simulated a 3D small cluster of mouse beta-cells via an extended stochastic Sherman-Rinzel-Keizer electrophysiological model. Progressive damage was modelled (0%, 31%, 69%, 84%, 94% and 98% of dead beta-cells) at different glucose concentrations, representing the different glycaemic states in the autoimmune progression towards type 1 diabetes (T1D). At 31% (normoglycaemia) and 69% (hyperglycaemia) of dead beta-cells, the system appeared to be biologically robust to maintain regular [Ca^{++}] oscillations guaranteeing an effective insulin release. Simulations at 84%, 94% and 98% of death beta-cells (severe hyperglycemia) showed that the complex periodic bursting pattern disappeared and [Ca^{++}] oscillations were absent. In such conditions insulin pulsatility is not expected to occur. Our results suggest that the islet tissue of beta-cells is biophysically robust enough to compensate high rates of cellular loss, a fact which is in agreement with in vitro animal experiments. The model indicates the necessity of maintaining glycaemia within physiological levels as soon as possible after diabetes onset in order to avoid a dramatic drop of [Ca^{++}] pulsatility and consequent insulin release. In the absence of glucotoxicity, any adjuvant therapy to cure this disease including immunomodulation or the regeneration of beta-cells can be more beneficial and hopefully contribute to impeding the destruction of any residual beta-cell still functioning.

Supported by: University Campus Bio-Medico, ICRANet & Centro Internazionale Studi Diabete

2534-PO

An Anti-Idiotypic Vaccine Prepared from Pancreatic Lymph Nodes Prevents T1DM in NOD Mice

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To develop an immune intervention that targets T-cell specific for a particular autoantigen we have evaluated anti-idiotypic vaccination in T1DM. The vaccine consists of antigen receptors specific for complexes of MHC with peptides derived from islet autoantigens. To obtain these receptors we used phage-display to enrich libraries for clones recognizing the desired MHC peptide complex. A critical point is the isolation of positive clones. Receptor libraries generated from mice immunized with the appropriate antigen can be successfully selected, whereas those generated from non-immunized mice may not yield positive clones. Translation of this approach is therefore limited since immunization for this purpose is not possible in humans. Alternatively, autoreactive T-cells in NOD mice and humans with T1DM are concentrated in pancreatic lymph nodes (pln), making pln (currently available from nPOD) suitable for construction of selectable libraries.

To test the feasibility of this approach we generated from pln of non-immunized NOD mice an antigen receptor library in the form Vα-linker-Vβ (TscFv for single chain fragment variable from T-cells). Since RegII and specifically its N-terminal fragment (NtfrII) acts as potential autoantigen in T1DM the library was enriched for clones recognizing complexes of $I\text{-}A^{g7}$ with NtfrII-derived peptides.

The enriched but not the non-enriched library distinguished NtfrII-pulsed from unpulsed NOD antigen presenting cells (APCs). Further characterization led to the isolation of S9/P2, a TscFv clone that recognized $I\text{-}A^{g7}$ bound to an NtfrII T-cell epitope capable of activating autoaggressive T-cells.

Vaccination of NOD mice with the selected TscFv library significantly delayed T1DM and B- but not T-cells from S9/P2-vaccinated mice were able to prevent disease in NOD-SCID mice when induced with diabetogenic $CD4^+$ NtfrII-specific T-cells but not when induced with T-cells of another specificity (BDC2.5). We conclude that selection of TscFv binding to HLA complexed with autoantigen-derived peptides from human pln libraries could yield antigens for effective anti-idiotypic vaccinations in humans at risk of T1DM.

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2535-PO

Anti-Autonomic Receptor Antibodies in Diabetic Orthostatic Hypotension

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Orthostatic hypotension (OH) is often associated with diabetes and has many causes. Very little new data exists relating to the pathophysiology of OH. We have recently identified the presence of agonist-like autoantibodies to beta-adrenergic and muscarinic receptors in a subgroup of patients with OH. These autoantibodies are frequently observed in patients with diabetes and concurrent cardiac diseases which often have associated OH. There is precedence for the pathological significance of such antibodies in cardiomyopathies. Using ELISA and bioassays, IgG from antibody-positive OH patients demonstrated significant capacity to activate their respective receptors. Patients with resting tachycardia had IgG with predominantly beta-adrenergic activity, while patients with a relative resting bradycardia and impaired pulse rate response in the face of OH had IgG with predominantly muscarinic activity. IgG from patients with antibodies to beta2-adrenergic and/or M3 muscarinic receptors produced an expected potent vasodilatation (5-20%) in the rat cremaster skeletal muscle arterioles which return to control after the infusion is finished, suggesting these antibodies may contribute to systemic vasodilatation. Three patients treated with combined M2/3 blockade (oxybutynin LR) showed decreased orthostatic symptoms and signs. These data support the hypothesis that these circulating agonistic autoantibodies cause or exacerbate OH by altering the postural cardiovascular response in these patients. These data will also be useful in identifying therapeutic strategies that target these autoantibodies.

Supported by: Diabetes CoBRE

2536-PO

WITHDRAWN

2537-PO

Development of a Tolerogenic Matrix Containing Autoantigens for the Prevention of Type 1 Diabetes

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Antigen specific immunomodulation remains an important goal in the treatment of type 1 diabetes (T1D). We hypothesized that T1D can be prevented through the use of an adjuvant modified matrix containing β cell autoantigens and a chemoattractant. In order to test this hypothesis, D, L-lactic acid-co-glycolic acid phagocytosable microparticles (MP) were used as a vehicle for delivery of insulin B chain: 9-23 (B9-23) and the adjuvants CpG or hemoglobin:haptoglobin (Hb:Hp). The MPs were then placed into a hydrogel containing GM-CSF as the chemoattractant. These formulations were injected subcutaneously into 12 week old female NOD mice (n=10/treatment group). Groups consisted of untreated mice, GM-CSF with empty MP, GM-CSF with

For author disclosure information, see page 785.

B9-23 MP, GM-CSF with CpG and B9-23 MP, GM-CSF with Hb:Hp and B9-23 MP, GM-CSF with CpG MP, and GM-CSF and Hb:Hp MP. Blood glucose was measured once a week until 32 weeks of age. Kaplan Meier analysis revealed a p-value of 0.0636 in terms of survival proportions in all groups. Unexpectedly, the groups treated with hydrogel mixed with GM-CSF as well as hydrogel mixed with GM-CSF and CpG MP had the highest survival rate (40% of survival proportions versus 0% of untreated animals). This implies that mobilization and/or activation may be more important in this setting, so we evaluated the capacity of NOD, NOR, and C57/BL6 splenocytes and bone marrow cells to migrate toward GM-CSF in an *in vitro* migration assay (n=3/strain). After 24 hrs, bone marrow cells and splenocytes from C57 and NOR showed a 100% increase in cell migration with GM-CSF. However, while no differences were seen in NOD bone marrow cell migration, splenocytes showed a migration defect ($p<0.05$). This implies that bone marrow mobilization may be necessary to improve this peripheral migration defect in NOD mice prior to immunomodulation therapies. In a previous study, we also noted a synergistic effect of G-CSF, which is another bone marrow mobilizing agent, and immunosuppressive agents (anti-thymocyte globulin or anti-CD3) in the reversal of T1D in NOD mice. A defect in peripheral migration should be considered in future human and NOD studies.

2538-PO

GADA and IA-2A Titers Correlated with Different Profile of Peripheral Cytokine and Chemokine at Type 1 Diabetes Diagnosis

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Humoral and cellular autoimmunity together with pro-inflammatory cytokines are implicated in the type1 diabetes (T1D) pathogenesis. Glutamic acid decarboxylase (GADA) and IA-2(IA-2A) auto-antibodies (auto-ab), two humoral markers of this disease, may be associated to different velocity progression to clinical diabetes. However the mechanism is not well known. The aim of this study was to evaluate the relationship between GADA and IA-2A (number and titers) with circulating cytokines and chemokines profile at diagnosis of T1D.

Thirty-five T1D (at diagnosis) were divided in two subgroups according to positive test for either (n=7; 14.8±5.6 years) or both auto-ab (GADA and IA-2A) (n=25; 12.5±4.8 years). Cytokines from undiluted serum were measured using inflammatory cytokine (IL-12, 1L-6, IL-1β, TNFα, IL-10) and chemokine (CXCL10, CXCL8, CXCL9, CCL2) bead array (CBA) Kits (Becton Dickinson, San Diego, CA, USA). GADA and IA-2A were determined by RIA (RSR Ltd). All cytokines and chemokines were significantly higher in T1D compared to health individuals control group ($p<0.001$), There were a negative association between GADA and CXCL10 ($r=-0.452$; $p=0.011$), CCL2 ($r=-0.656$; $p=0.000$) and daily insulin requirement ($r=-0.366$; $p=0.031$) whereas, IA-2A showed a negative correlation with IL-10 ($r=-0.385$; $p=0.027$) in the hole T1D group. The double auto-ab group showed higher IA-2A titer comparing to single positive ones (7.03 ± 10.0 U vs 1.06 ± 1.09 U; $p=0.03$). Considering the abnormal cytokine and chemokine sera levels $\geq$ 2 sd of those found in a health control group matched for age and BMI (n=25), the double auto-ab positive patients had higher levels for IL-12 (71.4% vs 42.9%; $p=0.05$) and CXCL10 (71.5% vs 57.1%; $p=0.05$). GADA is related to a better cytokine profile than IA-2A. The presence of double islet cell auto-ab (GADA plus IA-2A) or high IA-2A titer showed a positive association to inflammatory and negative to anti-inflammatory cytokines profile. These findings as confirmed by independent studies might collaborate to understand the relation among islet cell antibodies differences and T1D development and progression.

2539-PO

Immune Tolerance in Type 1 Diabetes

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Type 1 diabetes (T1D) is an autoimmune disease with progressive destruction of pancreatic beta cells. Fluctuations in glycemia and autoantibody levels in individuals at high-risk of developing T1D have been observed. Together with the presence of the honeymoon phase (HMP) in newly diagnosed T1D, this raises questions about the possible relapsing/remitting nature of the autoimmune process whereby periods of immune tolerance may occur along the disease pathway. It has also been shown that persistence of an antigen can lead to "adaptive immune tolerance (AIT)". Therefore, we tested the hypothesis that AIT occurs in new onset T1D in relation to the HMP. We used a T cell proliferation assay where CD4+CD45RO+ memory T cells are selected by magnetic beads, labeled with CFSE and then cultured in two protocols. The conventional assay was performed where cells were cultured for 7 days in the presence of defined islet cell HLA-restricted autoantigenic peptides (GAD, Insulin, IA-2) and responses determined. The reversion assay was performed where cells were cultured with no antigen for 7 days and peptides were added on day 7 and the assay read on day 14. Results were determined by flow cytometry and cell division index were calculated and analyzed for each analyte. The assay was carried out on 10 subjects in new-onset before and during the HMP. Conventional day 7 responses were minimal soon after diagnosis, but increased as patients entered the HMP. Reversion responses were prevalent both early and during HMP in a majority of subjects tested.

		Peptides	Subjects
Early (4-6 wks from onset)	Day 7	1/48	1/10
	Day 14	14/45	8/10
	p value	0.0001	0.0017
HMP (8-16 wks from onset)	Day 7	5/48	3/10
	Day 14	12/40	7/10
	p value	0.0205	0.0736
Total (combined data)	Day 7	6/48	5/17
	Day 14	26/45	14/17
	p value	< 0.0001	0.0019

In conclusion, absence of antigen for 7 days and then testing responses at day 14 demonstrates significant differences between conventional T cell proliferation and this reversion assay. We speculate that this procedure may be unmasking islet cell specific peptide responses that may provide evidence of in vivo tolerance that may define the immunological characteristics of T1D progression and the HMP.

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2540-PO

Islet Cell Autoantibody Reactivity on Pancreatic Tissue from Donors with Type 1 Diabetes

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Islet cell cytoplasmic autoantibodies (ICAs) are present in the majority of patients with type 1 diabetes (T1D) at clinical onset, and in individuals at risk for future development of T1D. Some human and mouse ICA autoantigens have been identified (glutamate decarboxylase, ICA512) but there is much heterogeneity in regards to antigens and cellular localization of different ICA species. Serum ICA is traditionally measured by indirect immunofluorescence on frozen human group O pancreas. In this study, ICA positive serum (160 Juvenile Diabetes Foundation Units) from a patient with longstanding T1D was applied to group O pancreas sections from 7 donors (n=2 T1D, n=3 type 2 diabetes, n=2 without diabetes) in order to compare ICA reactivity and localization. Previous immunohistochemical characterization revealed that pancreata from both T1D donors contain insulin-negative/glucagon-positive islets and all pancreata from donors with type 2 diabetes (T2D) contain insulin-positive/glucagon-positive islets. Surprisingly, islet immunofluorescence was observed with the ICA positive serum on sections from all donors. Islets from the nondiabetic and T2D donors were easily identified. In contrast, islets were more difficult to identify in longstanding T1D patients due to loss of insulin, less well-defined endocrine-exocrine interface, irregular islet shape, and disrupted islet tissue morphology. Despite these differences, insulin-negative islets from T1D donors were still ICA reactive, indicating ICA autoantigens in this patient were not unique to beta cells but also expressed in other islet cell types, suggestive of a generalized islet inflammation in T1D despite preferential loss of beta cells. Among the T2D donors, islet immunofluorescence was most intense on sections from the 33 year-old insulin-dependent donor (C-peptide 22.4 ng/ml) with diabetes duration of 17 years, suggesting a relationship between higher beta cell activity and increased expression of autoantigens. Ongoing studies will attempt to correlate immunohistochemical findings with differences in ICA reactivity in order to better define the cell types and their respective autoantigens involved in T1D pathogenesis.

2541-PO

Pancreatic Endocrine Dysfunction in Subjects with IgG4-Related Disease without Autoimmune Pancreatitis

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IgG4-related disease (G4RD) is characterized by IgG4-bearing plasma cells infiltration into the involved organs with elevated serum IgG4 level.

Autoimmune pancreatitis (AIP), one disease entity of G4RD, has been recognized as exacerbating cause of blood glucose control in type 2 diabetic subjects. In a pilot study, we preliminarily found that G4RD very frequently accompanied diabetes and glucose intolerance, even without AIP. However, to date, there is no compelling evidence on the association of diabetes and glucose intolerance with G4RD without AIP. Therefore, the aim of this study was to characterize the pancreatic endocrine function in G4RD subjects.

Twenty-nine subjects were diagnosed as G4RD with histopathology and images. To evaluate pancreatic endocrine function, 75g oral glucose tolerance tests (OGTT) and arginine tolerance tests (ATT) were performed in the subjects.

Clinical features of the subjects were as below; male/female 15/14, age 62±9 years old, BMI 23.5±4.3kg/m^2, IgG 2493±1108 mg/dL, IgG4 662±470 mg/dL and HbA1c 6.3±1.7%. No subject without AIP had any morphologic abnormality in pancreas. OGTTs on 20 subjects elucidated that 11 were diabetes mellitus (DM), 4 were impaired glucose tolerance (IGT), and 5 were normal glucose tolerance (NGT). Their HOMA-IR, HOMA-β and insulinogenic index were 2.5±1.7, 77.4±38.3 and 0.5±0.4, respectively. Among 14 subjects (DM 9, IGT 2, NGT 3), who underwent ATT, 10 showed glucagon hyperreactivity (peak level of IRG ≥ 300 pg/mL).

Five subjects were treated with glucocorticoid (GC); initial dose of prednisolone was 27.8±8.9mg/day. Two subjects with GC were also prescribed with anti-DM medications, and their HbA1c levels have not changed through GC therapy.

These results indicate that there is a high frequency of pancreatic endocrine dysfunction in G4RD without AIP. Endocrine function of G4RD is characterized by preserved insulin secretion and glucagon hyperreactivity. Both of them would have been recovered if strict blood glucose control with multiple insulin injection had been introduced in the early stage of diabetes. Thus we emphasize the importance of earlier evaluation of pancreatic endocrine function in G4RD subjects.

2542-PO

Rapid Enrollment to GAD65-Alum Trial in Recent Onset Type 1 Diabetes Including Subjects as Young as Age 3

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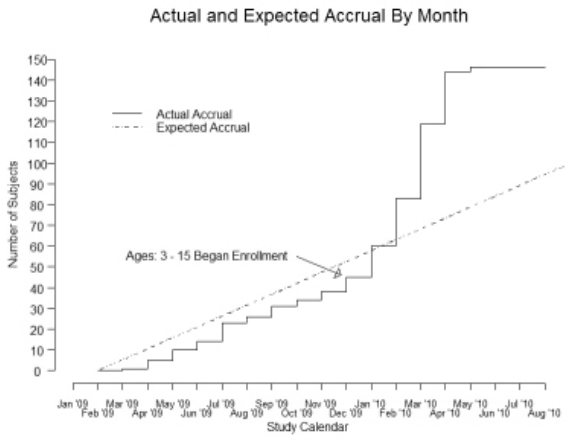
Type 1 Diabetes TrialNet is conducting a trial of glutamic acid decarboxylase (GAD) immunization in new onset type 1 diabetes (T1D) patients to determine if this therapy can slow the loss of β cell function. T1D patients aged 3-45 diagnosed within 3 months, positive for GAD antibodies (Ab) and with mixed meal stimulated C-peptide ≥ 0.2 pmol/mL were randomized to receive 3 injections of GAD-Alum (GAD/Al×3), 2 injections of GAD-Alum and 1 of Alum (GAD/Al×2 + Al) or 3 injections of Alum (Al×3). The primary endpoint, area under the curve for stimulated C-peptide at one year, will be reached in May 2011. Secondary endpoints are HbA1c, insulin dose, safety and immunologic mechanisms. Transcriptional and cytokine profiling, as well as T cell response, repertoire and specificity studies are being performed by five blinded laboratories to assess evidence of immune effects. Enrollment was staggered with those ≥ 16 years being randomized initially. Once enrollment was opened to subjects 3 and over, the trial filled rapidly, within an additional 5 months. 280 subjects were screened for eligibility. The most common reason for ineligibility was lack of GAD antibodies (72%). 276 had GAD Ab measured: 36% were GAD Ab negative, 41% of ages 3-10, 33% of 11-15 and 33% of ≥ 16. 146 subjects were randomized: 28%, 27% and 45% were ages 3-10, 11-15 and ≥ 16, respectively. Unique aspects of this trial include inclusion of very young subjects < 6 years of age with rapid enrollment of this group, suggesting acceptability of trials in very young new onset subjects.

Baseline Factors	Treatment Group		
	Al×3 N=49	GAD/Al×2 + Al N=49	GAD/Al×3 N=48
Mean Age Range	15 4 - 45	14 3 - 45	14 3 - 45
Mean days from diagnosis to trial entry Range	86.7 42 - 106	87.0 47 - 104	83.9 45 - 105
Mean AUC C-peptide (pmol/mL)	0.722	0.697	0.768
Mean HbA1c (%)	6.4	6.6	6.6
Mean insulin dose (units/kg)	0.40	0.32	0.38



ADA-Funded Research

2543-PO

Stiff Person Syndrome in Type 1 Diabetes Patients

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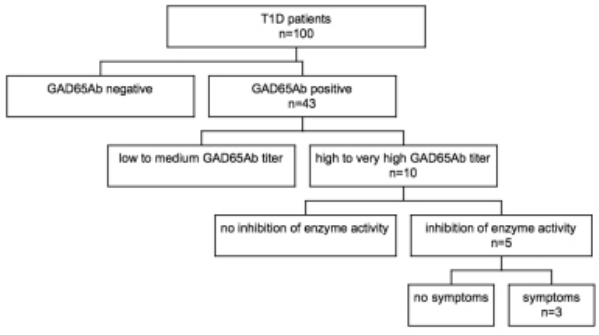
SPS is a rare neurological autoimmune disease characterized by autoantibodies (Ab) to proteins involved in neurotransmission. A major autoantigen is the 65kDa isoform of glutamate decarboxylase (GAD65), one of two enzymes catalyzing the formation of the inhibitory neurotransmitter GABA. The variability of symptoms between individuals may cause a failed diagnosis of milder cases. Our goal was to determine the prevalence of SPS in a cohort of T1D patients (n=100) randomly recruited from an academic diabetes practice. T1D patients often have circulating GAD65Ab, but only 1/10,000 T1D patients are diagnosed with SPS, possibly due to significant differences in GAD65Ab titers and inhibition of GAD65 enzyme activity. We used these 2 criteria (titer and enzyme inhibition) to identify individuals with SPS in this cohort. Information of the patients' age (median:42 years), duration of T1D (median:25 years), gender (56% female), and general health were available.

43% of the patients had detectable GAD65Ab titers (median:400U/ml, range:142-250,000U/ml) (range in healthy controls:0-135U/ml). GAD65Ab titers in 10 patients exceeded the 90th percentile of the cohort (2,000U/ml) and GAD65Ab in 5 of these patients inhibited the enzyme activity significantly (by 34-55%). Three of these patients complained of muscle stiffness and pain. Based on the observation that GAD65Ab in SPS patients are present both in circulation and the cerebrospinal fluid (CSF), we suggest that GAD65Ab are absent in the CSF of the 2 asymptomatic patients.



We conclude that SPS is more frequent in T1D than is currently recognized, and recommend that a diagnosis of SPS should be considered in T1D patients with unexplained muscle stiffness and pain. **ADA-Funded Research**

For author disclosure information, see page 785.

ADA-Funded Research

TRANSPLANTATION

2544-PO

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

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To observe the curative effectiveness of allogeneic bone marrow mesenchymal stem cells (BMSCs) transplantation in diabetic rats and the possible mechanism. BMSCs were isolated from allogeneic bone marrow of SD rat by adhesive screening method and cultured in vitro. The second passage of BMSCs was transfected with Ad-GFP. BMSCs that stably expressed GFP were obtained before transplantation. Streptozotocin-induced diabetic rats (plasma glucose≥16.7mmol/L) were divided at random into untreated group (UTG) and BMSCs transplantation group (TPG). BMSCs were transplanted into allogeneic diabetic rats of TPG by tail vein injection (0.4ml, $10^{6\text{-}7}$/ml). After the 2 weeks transplantation, the levels of plasma glucose, HbA_1c and insulin were determined. The pancreas was dissected out for pathological sections. Insulin reaction with immunohistochemistry was observed. Fluorescent microscopy was used to observe the expression of GFP labled cells in the pancreas. The result showed that levels of plasma glucose and HbA_1c were higher obviously in UTG than in normal control group (NCG), and insulin level lower (P<0.001). The levels of plasma glucose and HbA_1c were lower in TPG than in UTG(P<0.05), and insulin level higher(P>0.05).

Table 1. Levels of plasma glucose,HbA_1c and insulin in each group rats (means±*SD*)

	n	plasma glucose(mmol/L)	HbA_1c	insulin (mU/L)
NCG	5	7.97±1.00	9.15±0.82	33.68±6.21
UTG	6	27.96±1.87 Δ	22.51±2.99 ♦	11.57±3.25 ♦
TPG	9	23.63±6.42 &	18.26±3.96 ♥	13.89±7.89 &

Δ P<0.01,♦ P<0.001vs NCG; & P>0.05, ♥ P<0.05 vs UTG

The numbers of beta-cells of the UTG obviously decreased and the insulin reaction showed negative results. The numbers of beta-cells of the TPG increased, and the insulin reaction showed positive and strong positive results. The cells of GFP were observed in the excrine tissues of pancreas, but not observed in the pancreas islet.

It was concluded that BMSCs transplantation could engraft into pancreas tissues of diabetic rats, depress the levels of plasma glucose and HbA_1c, accelerate the synthesis of the insulin, might promote endogenous regeneration of islet in diabetic rats.

2545-PO

Cultured Human Islets with Allogeneic Bone Marrow (BM) Significantly Reduce Lymphocyte Proliferation In Vitro

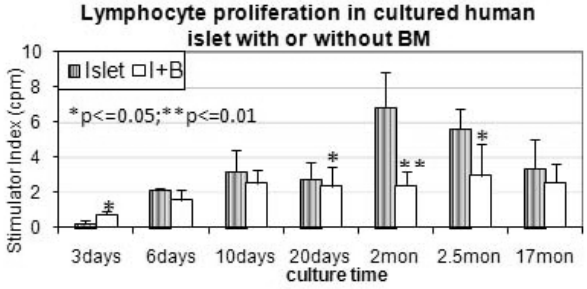
LUGUANG LUO, FANG XIONG, JOHN ZQ LUO, *Providence, RI*

Objective. Human islets have been shown to lose function shortly after transplantation in vivo, which has greatly impeded islet transplantation. Immuno-rejection plays a critical role in hindering islet function. Bone marrow (BM) and its derived stem/progenitor cells have demonstrated the ability to support human islet regeneration by promoting angiogenesis and inhibiting cytokine release. This may prevent immuno-reaction (IMRA) in vivo. In this study, we explored whether cocultured human islets with allogeneic BM, mesenchymal (MSC) and endothelial cells (EDC) alter the tissue's IMRA through evaluating human lymphocyte proliferation (LP) in vitro.

Research Design and Methods. We co-cultured BM ($1x10^6$/ml), MSC and EDC ($10x10^4$) with 100 islets (IEQ/ml). Cultured medium was collected accordingly and used to test human LP. The incorporated ^{3}H-thymidine activity was determined as counts per minute (CPM) using a β-counter for analyzing LP. Human islet (I) β-cell function was evaluated by Human insulin ELISA kits.

Results. Significant differences were observed in LP between I and I+BM cultures on day 3 (0.292±0.16 vs. 0.761±0.21 p< 0.05), day 60 (6.87±2.01 vs. 2.39±0.85 p<0.01) and day510 (3.38±1.69 vs. 2.56±1.07). β-cell insulin release was also significantly different between I and I+BM groups (6901±845 vs. 11506±2443 on day 3, 916±468 vs. 11941±3091 on day510, μU/ml p<0.01). However, no significant differences were found between the I, I+MSC or I+EDC groups.

Conclusions. Results suggest that allogeneic BM retains low levels of LP in human islet co-cultures, thus having the potential to sustain islet function by reducing immune reaction. This may lead to new techniques to reduce immunorejection in human islets in vivo.



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2546-PO

Duration and Timing of Glucocorticoid Treatment Independently Affects on the Expansion and Transdifferentiation of Porcine Neonatal Pancreas Cell Clusters (NPCCs)

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We underwent this study to determine the effects of duration and timing of glucocorticoid treatment on expansion and differentiation of porcine neonatal pancreas cell clusters (NPCCs) into β-cells and also to elucidate the intracellular action mechanism. After transplantation of 4,000 IEq porcine NPCCs beneath the renal subcapsular space of normoglycemic nude mice, dexamethasone (Dx: 1 mg/kg) or vehicles were injected daily for 10 weeks. For observing the effect of timing or duration of Dx treatment, we injected the Dx only at first 2 weeks or last 8 weeks during 10 weeks period after transplantation. The relative volume and absolute mass of β-cell in the grafts were significantly lower in Dx treatment group than those of the control group at 10 weeks after transplantation (relative volume of β-cells: 22 % *vs.* 35.3 %, β-cell mass: 0.96±1.2 *vs.* 2.23±5.6, Dx *vs.*control group). Total graft masses were also significantly decreased in Dx treatment group (Dx *vs.* control: 4.36±5.4 vs 6.32±1.3). The area of duct cyst and β-cell mass in the grafts showed positive and negative relations with the duration of Dx treatment respectively. However total graft mass were significantly reduced by only initial 2 weeks treatment of Dx. The expressions of Pdx-1, HNF-3β were significantly down-regulated while PGC-1α expression was increased by Dx treatment in the grafts and monolayer cultured porcine NPCCs. Pancreatic duct cell apoptosis was significantly increased by Dx treatment while proliferation rate was not changed. All together transdifferentiation of the porcine NPCCs into β-cells are influenced by duration of Dx treatment which might be resulted by suppression of pancreas key transcription factors and PGC-1α plays an important role in the expansion and transdifferentiation of porcine NPCCs. While the initial 2 weeks after transplantation of porcine NPCCs is a critical period for determining the final β-cells mass in the grafts.

2547-PO

Early Detection of Alterations in beta-Cells Function and Insulin Sensibility in Patients with Type 1 Diabetes and Kidney and Pancreas Transplant

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The insulin resistance and decreased beta-cell function are key elements in the pathogenesis of post-transplant diabetes. There are not early markers of pancreatic tissue rejection, being hyperglycemia a late sign of irreversible rejection. Have indices that allow us to calculate beta-cells functionality failures in the early stages can help develop strategies for histological confirmation and adjustment of immunosuppressive therapy. We study 12 healthy subjects and 9 patients one year after underwent kidney and pancreas transplant. These patients had type 1 diabetes of long duration with terminal renal failure. Baseline levels of glucose (G0), insulin (I0), C-peptide (CP0) and HbA1c were analyzed. Glucose was also analyzed in the patients by a Continuous Glucose Monitoring System (CGMS). We calculated simple indexes of insulin sensitivity (HOMA-IR) and beta-cells function (HOMA-β). Insulin sensitivity is also estimated by IVGTT (SI). Others indices associated with the secretion of insulin are calculated from IVGTT: area under the curve (AUC) of insulin and C-peptide between 0-10 minutes ($AUC\text{-}I_{0\text{-}10}$ y $AUC\text{-}PC_{0\text{-}10}$). Data from controls and subjects with pancreas

and kidney transplant (PKT) (Control vs. PKT): Insulin (mIU/mL): 8.8±4.1 vs. 20.5±10.4, P<0.001; C-peptide (ng/mL) 2.4±1.7 vs. 2.7±0.5 P=0.069; AUC-$PC_{0\text{-}10}$: 12.4±7.1 vs. 22.6±12.3, P=0.023; HOMA-β: 25.2±13.4vs. 46.4±20.7, P=0.015; SI: 7.5±5.5 vs. 5.8±2.3, P=0.824: HOMA-IR: 1.89±1.06 vs. 4.48±2.37, P=0.001. AUC-$I_{0\text{-}10}$ significantly correlated with SI (r=-0.488, p=0.029). AUC-$PC_{0\text{-}10}$ significantly correlated with CGMS (r=0.719, p=0.045), HbA1c (r=0.795, p=0.018) and SI (r=-0.445, p=0.049). In conclusion, there are differences in insulin resistance and beta-cells function between controls and patients with kidney and pancreas transplant and with normal plasma glucose. AUC-$PC_{0\text{-}10}$ shows a significant correlation with clinical parameters such as mean plasma glucose and HbA1c.

2548-PO

Effect of Intrapancreatic Islet Transplantation Combined with Bone Marrow Mesenchymal Stem Cells and Islet Neogenesis-Associated Protein Treatment on Diabetic Mice

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Pancreatic islets implanted heterotopically become poorly revascularized following transplantation. The aim of this study was to investigate the effects of combination treatment of intrapancreatic islet transplantation, bone marrow mesenchymal stem cells (MSC) transplantation and islet neogenesis-associated protein (INGAP) injection on streptozotocin induced diabetic mice. We divided streptozotocin induced diabetic C57BL/6 mice into 5 groups: group 1 was untreated control group, group 2 received 300 islet equivalent(IEQ)-transplanted in pancreas, group 3 received MSC transplanted by tail intravenous injection, group 4 received INGAP treatment by daily intraperitoneally injection for 30 days, and group 5 received combination treatment. Non-fasting blood glucose was assayed once every two days. At day 35, insulin, glucagon and Pdx-1 expression in pancreas were measured by immunohistochemistry. Real time-PCR was performed to test Pdx-1, Glut-2 and VEGF mRNA expression in pancreas. Combination treatment of islet transplantation, MSC transplantation and INGAP injection significantly decreased blood glucose compared with other groups, which only reduced blood glucose partially. No mice performed pancreatitis after transplantation. Immunohistochemistry of pancreas revealed that intrapancreatic islet grafts were survived after 35 days both in islet transplantation group and combination treated group. The expression of VEGF and Glut-2 in pancreas were higher in combination treated group, and the expression of PDX-1 was higher in MSC transplanted group. Our data suggest that intrapancreatic islet transplantation combined with MSC transplantation and INGAP injection reverses hyperglycemia through enhancing islet vascularization and promoting islet neogenesis.

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2549-PO

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2550-PO

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2551-PO

Oral Glucose Tolerance Test in the Study of Glucose Alterations in Patients Undergoing Liver Transplantation

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Liver cirrhosis is associated with insulin resistance and glucose intolerance. Since these patients have usually normal fasting glucose (FG) and low glycated haemoglobin (HbA1c), these alterations may remain undiagnosed if an oral glucose tolerance test (OGTT) is not performed. The objective is to characterize the glucose alterations in patients with advanced cirrhosis using an OGTT, and to observe if these alterations improve after liver transplantation (LT). Descriptive study of 22 patients (90% men) with normal FG undergoing LT from march to june 2010. An OGTT was performed before and 4 months after LT. HbA1c and insulinemia after OGTT were determined. Area under the curve of OGTT (AUC) was calculated by trapezoidal method. Mean age was 55.4 (8.2) years. Mean FG was 91.1 (18.9) mg/dl and HbA1c 4.8 (0.6)%. When OGTT was performed 75.6% of patients were diagnosed of diabetes (blood glucose ≥200 mg/dl 2 hours after OGTT) and 9.8% of impaired glucose tolerance (blood glucose >140 and <200 mg/dl). After LT, 2 patients (9.1%) had diabetes diagnosed by FG. Among the patients who underwent OGTT after LT (20), 40.3% had diabetes and 26.3% presented impaired glucose intolerance. The percentage of diabetes diagnosed either by FG or by OGTT at 4 months after LT was 46.7%. HbA1c after LT increased to 5.7 (0.9)% (p<0.01). Pretransplant HbA1c did not correlate with pretransplant AUC. Postransplant HbA1c correlated with postransplant AUC. Table 1 shows OGTT before and after LT. A significant decrease in insulinemia after

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transplant was observed (table 2). In conclusion, there is a high prevalence of diabetes in patients with advanced cirrhosis that could be undiagnosed if the diagnosis is only based on FG and HbA1c. Impaired glucose metabolism seems to improve after transplantation, perhaps it is due to an improvement in insulin resistance.

OGTT (mg/dl)	PRETRANSPLANT	POSTTRANSPLANT	p
0´	86 (5.9)	88.2 (13.6)	0.6
60´	198.3 (32.9)	173.9 (33.6)	0.05
120´	224.7 (60)	174.8 (61.5)	<0.05

insulinemia after OGTT	PRETRANSPLANT	POSTTRANSPLANT	p
0´	14.2 (2.3)	9.6 (8.1)	0.2
60´	74.9 (22.6)	27.6 (13.5)	<0.01
120´	179.7 (78.3)	67.8 (47.4)	<0.05

INSULIN ACTION—GLUCOSE TRANSPORT

2552-PO

The In Vitro Effects of Saturated and Unsaturated Fatty Acids on Insulin Stimulated Glucose Metabolism of Myocytes and Adipocytes

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High levels of plasma free fatty acids has been associated with insulin resistant states such as obesity and development of type two diabetes.

This study aims to determine the *in vitro* effect of the fatty acids (FAs) palmitate, oleate, omega 3 and omega 6 on insulin stimulated glucose metabolism in myocytes and adipocytes.

C2C12, L8 and 3T3-L1 cells were cultured in DMEM supplemented with 10% fetal calf serum. Myotubule formation was induced in the C2C12 and L8 cells. 3T3-L1 cells were transformed into adipocytes with IBMX and insulin. Myocytes and adipocytes were exposed to DMEM containing 5.5 or 20 mM glucose and 0.75 mM FA for 24 hours. Glucose uptake was determined with ^{3}H-2-deoxyglucose. Glycogen and G6P were determined calorimetrically. Glucose oxidation was determined by $^{14}CO_2$ release from glucose D-[^{14}C (U)].

Palmitate decreased basal and insulin stimulated glucose uptake in C2C12 (2.83 and 2.83 fold) and L8 myocytes (2.17 and 2.12 fold). Oleate had no effect on basal glucose uptake in myocytes but decreased insulin stimulated glucose uptake in C2C12 cells (1.83 fold). Omega 3 had no effect on both basal and insulin stimulated glucose uptake while omega 6 decreased basal and insulin stimulated glucose uptake in L8 cells (1.79 and 2.1 fold). In C2C12 and L8 cells palmitate reduced intracellular G6P but not glycogen. Glucose uptake in 3T3-L1 adipocytes were unaffected by the FAs at 5.5 mM glucose but was reduced by palmitate at 20 mM glucose. Palmitate reduced glucose oxidation in L8 myocytes and adipocytes at 5.5 mM glucose. At 20 mM glucose all FAs reduced insulin stimulated glucose oxidation in all three cell lines.

In conclusion, glucose uptake and utilization in myocytes, especially at high (20 mM) glucose concentrations, are more sensitive to FA exposure than 3T3-L1 adipocytes.

INSULIN ACTION—INSULIN RESISTANCE IN VITRO

2553-PO

Acute Induction of Insulin Resistance with Glucosamine Infusion Increase Pancreatic Islet Blood Flow in Anesthetized Rats

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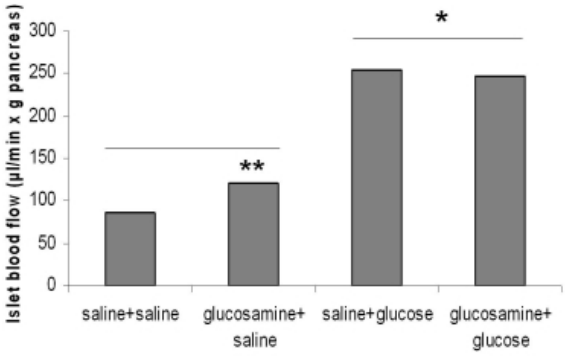
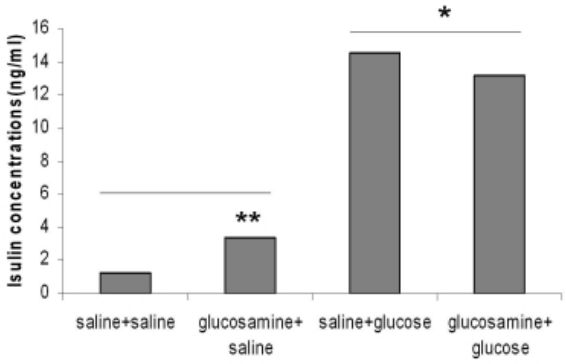
Conditions with increased demands for insulin secretion, including experimental type 2 diabetes, is associated with increased pancreatic islet blood perfusion. The mechanisms are mainly neural, but also locally formed products, such as nitric oxide, are involved. To what extent high blood flow affects islet function has been difficult to study.

The aim of this study was to evaluate if an acutely increased peripheral insulin resistance induces an islet blood flow increase. This would enable us to further study the mechanisms and functional consequences of this on islets.

Anesthetized Sprague-Dawley rats were infused with glucosamine (6mg/kg/h) or saline for 2 hours. After 120 min serum insulin concentrations were almost doubled in the normoglycemic rats, whilst no further potentiation was seen in the hyperglycemic rats. Pancreatic islet blood flow, measured with a microsphere technique, was higher in the control glucosamine-infused rats than in the control rats, whilst the other organ blood flow values were unaffected. In hyperglycemic rats the normal glucose-induced increase in islet blood flow was unchanged. When glucosamine was administered into the arterioles of isolated pancreatic islets during normoglycemic conditions an 8% vasodilation was seen.





Acute glucosamine-infusion increases serum insulin concentrations suggesting the presence of an insulin resistance. This leads to a selective increase in pancreatic islet blood flow, which is probably mainly due to a direct vasodilator effect.

2554-PO

High Fat Diets Leads to Insulin Resistance and Suppresses PGC-1α Expression by P38-MAPK Pathway in Skeletal Muscle Cells

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High fat diet could lead to insulin resistance and down-regulate PGC-1α expression in skeletal muscle of rats, however, the role of MFN2 in insulin resistance and the mechanism by which high fat diet regulates the expression of PGC-1α should be further elucidated.

Male wistar Rats were given basal feed (NC group, n=16) or high-fat feed (HF group, n=16) for 8 weeks, the fasting blood-glucose (FBG), insulin (INS), serum triglyceride (TG), serum total cholesterol (TC), and FFA were assayed. Meanwhile, the glucose infusion rate (GIR) was determined by hyperinsulinemic euglycemic clamp. The expression of PGC-1α in quadriceps femoris was measured by RT-PCR and Western blot. C2C12 cells were incubated with palmitic acid for different times, with or without P38 inhibitor SB203580, the expression levels of PGC-1α, ERK, p-ERK, JNK, p-JNK, P38, p-P38 were assayed by western-blot. The results showed: 1. The values of FBG, INS, FFA and TG were higher, but GIR was decreased in rats of HF group compared with NC group. 2. The expression of PGC-1α decreased in HF group and palmitic acid incubated C2C12 cells compared with control group. 3. Without P38 expression change, the p-P38 level increased in palmitic acid incubated cells, while the expression levels of ERK, P-ERK, JNK, and P-JNK showed no marked change. 4. Palmitic acid incubation down-regulated PGC-1α expression in C2C12 cells, while the effect was antagonized by p38 inhibitor.

In conclusion, High fat diet could lead to insulin resistance, as maybe associated with down-regulation of PGC-1α expression by P38-MAPK pathway in skeletal muscle cells.

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