Type 1 Diabetes: Clinical and Experimental

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The rising incidence in type 1 diabetes (T1D) continues to stimulate experimental and clinical studies aiming at understanding the pathogenesis of the disease and developing new preventive and therapeutic strategies. This trend in T1D incidence appears to be too rapid to be simply justified by the genetic background, whereas it seems more likely to be due to changes in environmental factors [1], such as enteroviruses, early life nutrition and vitamin D deficiency. Interestingly, over the last year there has been a particular focus in trying to understand whether these factors directly trigger the autoimmune process or mainly influence the progression from autoimmunity to clinical manifest T1D. With regard to genetic studies in T1D, a further step after the genome-wide association (GWA) era has been the attempt to integrate GWA data with gene expression profiles in order to translate genetic associations into biological function, as well as the assessment of the interaction between genetic and environmental factors to fully characterize the complex etiology of T1D.

Prevention is a fundamental issue in the context of diabetes and several ongoing studies are assessing the role of dietary interventions started early in life. Studies focusing on a way of improving the success of islet transplantation and on the development of new non-invasive biomarkers of insulinitis offer new hopes and food for thought.

Last but not least, when considering childhood-onset T1D, one aspect which needs to be carefully considered is related to its acute and chronic complications, which contribute to the burden of the disease. New data based on long-term follow-up of a cohort with childhood-onset T1D have been published in 2010–2011 and indicate that mortality among people with T1D is still higher than in the general population. A better understanding of the pathogenesis of T1D complications represents the starting point for new intervention strategies which could improve the prognosis of patients. All the above-mentioned aspects are the focus of the articles chosen for this year's chapter on T1D.

Clinical trials, new treatments

Early interventions to prevent autoimmunity

Dietary intervention in infancy and later signs of β -cell autoimmunity

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N Engl J Med 2010;363:1900–8.

Background: Early exposure to complex dietary proteins may increase the risk of β -cell autoimmunity and T1D. In the present study the effect of supplementing breast milk with highly hydrolyzed milk formula on the incidence of diabetes-associated autoantibodies was assessed in children with genetic susceptibility.

Methods: In a double-blind, randomized trial, 230 infants with HLA-conferred susceptibility to T1D and at least 1 family member with T1D were assigned to receive either a casein hydrolysate formula (intervention) or a conventional, cow's-milk-based formula (control) during the first 6–8 months of life. T1D autoantibodies were analyzed during a median observation period of 10 years. Children were monitored for incident T1D until the age of 10 years.

Results: The unadjusted hazard ratio (HR) for positivity for one or more autoantibodies in the intervention formula group was 0.54 (95% confidence interval (CI) 0.29–0.95), compared with the control group, and the adjusted HR for an observed difference in the duration of exposure to the study formula was 0.51 (95% CI 0.28–0.91). The unadjusted HR for positivity for ≥ 2 autoantibodies was 0.52 (95%

CI 0.21–1.17), and the adjusted HR was 0.47 (95% CI 0.19–1.07). The rate of reported adverse events was similar in the two groups.

Conclusions: Dietary intervention during infancy appears to have a long-lasting effect on markers of β -cell autoimmunity.

There is increasing evidence suggesting that β -cell autoimmunity is a phenomenon deriving from the interaction of genetic and environmental factors, which may be induced early in life and potentially even during prenatal life [2]. Animal and human studies have shown that reduced breastfeeding and an early introduction of complex dietary proteins can influence the development of β -cell autoantibodies [3]. These observations represented the starting point for the present study, which assessed whether the composition of two different formulas given to infants was associated with seroconversion to T1D autoantibodies. In a randomized double-blind study, 230 at term newborn with genetic susceptibility were assigned to receive either an extensively hydrolyzed casein-based formula (intervention formula) or a control formula, composed of 80% intact milk protein and 20% hydrolyzed milk protein. Interestingly, the intervention formula was associated with a significant reduced risk of seroconversion to one or more autoantibodies: 17% of babies in this group developed at least one autoantibody vs. 30% in the control formula group. Although there were some differences in the time when the two formulas were introduced and in the duration of their implementation, the results remained consistent after adjusting for these potential confounders. The mechanisms behind the effect of the intervention formula is not clear, although it has been hypothesized that this could be due to effects on the gut and the microbiota, such as reduced gut permeability, modification of the gut microflora, induction of the maturation of regulatory T cells in the gut-associated lymphoid tissue, as well as to the elimination of early exposure to bovine insulin [3]. Although further studies are required to confirm the findings of the present study, these results highlight the feasibility of preventive dietary interventions started early in life as a tool to manipulate β -cell autoimmunity. Early nutritional interventions represent indeed an appealing strategy for preventing T1D because they could be easily applicable as a public health strategy.

New concerns

Looking for an explanation for the increasing T1D incidence?

Accelerated progression from islet autoimmunity to diabetes is causing the escalating incidence of type 1 diabetes in young children

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J Autoimmun 2011;37:3–7

Background: This study was performed to determine whether the incidence of islet autoimmunity or the rate of progression from autoimmunity to diabetes onset has changed over a 20-year period in children genetically predisposed to T1D.

Methods: Between 1989 and 2010, children who were first-degree relatives of patients with T1D and who were born in Germany were prospectively followed from birth without intervention. A total of 324 children (BABYDIAB study) born between 1989 and 2000 and 216 children (TEDDY study) born between 2004 and 2010 with matched HLA genotypes were recruited before age 3 months and included for analysis. Children were followed for the development of T1D autoantibodies and for progression to diabetes.

Results: The cumulative frequency of T1D by age 4 years was 2.5% (95% CI 0.8–4.2%) in BABYDIAB children and 6.2% (95% CI 2.3–10.1%) in TEDDY children ($p = 0.03$). The cumulative frequency of islet autoantibodies by age 4 years was similar in the children from both studies (11.3 vs. 13.9%). Progression to diabetes from the appearance of islet autoantibodies was markedly increased in children from the more recently recruited TEDDY cohort than in BABYDIAB children: 50% progression within 9.6 vs. 85.2 months; $p = 0.009$. This difference persisted even when children were further

selected on the basis of high-risk HLA genotypes or the development of autoantibodies to multiple islet antigens ($p = 0.01$).

Conclusions: The recent increasing incidence of T1D in young children could be due to weakening of mechanisms that normally regulate autoimmune destruction of islet β cells.

The escalating increase in new cases of T1D observed during the last decades, particularly in children younger than 5 years, continues to stimulate studies aiming at understanding the underlining mechanisms. One important issue to clarify is whether this trend reflects an increase in the incidence of early autoimmunity against the pancreatic β cells or a faster progression from the appearance of autoimmunity to clinical manifest T1D. This was the key aspect investigated in the study reported by Ziegler et al., where children at risk of developing diabetes, as defined by having a first-degree relative with T1D and a high-risk HLA haplotype, were followed from birth up to 20 years. These children were recruited to two large ongoing studies, BABYDIAB and TEDDY, which have been assessing autoimmunity and progression to T1D in children with an increased genetic susceptibility. For the BABYDIAB cohort, recruitment of at risk newborn was conducted between 1989 and 2000, whereas TEDDY represents a more recent cohort established between 2004 and 2010. One of the main study findings was that the percentage of positivity for autoantibody by age 4 was similar in both cohorts: 11.3% in BABYDIAB vs. 13.9% in TEDDY. In contrast, the time for progression to T1D, calculated from the first appearance of autoantibodies, was more rapid in the more recent TEDDY cohort than in the BABYDIAB cohort: 50% progression occurred within 9.6 months in the TEDDY vs. 85 months in the BABYDIAB children. This suggests that the current increasing incidence of diabetes in young children could be related to mechanisms implicated in the progression from autoimmunity to T1D progression, which therefore represent specific targets for the development of future interventions.

New concerns

Discovering the vascular effects of hypoglycemia

Effects of acute hypoglycemia on inflammatory and pro-atherothrombotic biomarkers in individuals with type 1 diabetes and healthy individuals

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Diabetes Care 2010;33:1529–35.

Background: Recent large randomized trials have linked adverse cardiovascular and cerebrovascular events with hypoglycemia. The aim of this study was to determine whether 2 h of moderate clamped hypoglycemia could decrease fibrinolytic balance and activate pro-atherothrombotic mechanisms in individuals with T1D and healthy individuals.

Methods: 35 healthy volunteers (19 male, age 32 ± 2 years, BMI 26 ± 2 kg/m², A1C $5.1 \pm 0.1\%$) and 24 with T1D (12 male, age 33 ± 3 years, BMI 24 ± 2 kg/m², A1C $7.7 \pm 0.2\%$) were studied during either a 2-hour hyperinsulinemic euglycemic or hypoglycemic clamp or both.

Results: Insulin levels were similar (602 ± 44 pmol/l) in all four protocols. Glycemia was equivalent in both euglycemic protocols (5.2 ± 0.1 mmol/l), and the level of hypoglycemia was also equivalent in both T1D subjects and healthy control subjects (2.9 ± 0.1 mmol/l). Plasminogen activator inhibitor (PAI-1), vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM), E-selectin, P-selectin, interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), and adiponectin responses were all significantly increased ($p < 0.05$) during the 2 h of hyperinsulinemic hypoglycemia as compared with euglycemia in healthy control subjects. All measures except PAI-1 were also found to be increased during hypoglycemia in T1D.

Conclusions: Acute hypoglycemia can have significant vascular effects including activation of prothrombotic, proinflammatory, and pro-atherogenic mechanisms in individuals with T1D and healthy individuals.

The negative effect of acute and chronic hyperglycemia on vascular complications of T1D is well known and it is related to the activation of several pathways: inflammation, oxidative stress, prothrombotic and anti-fibrinolytic factors [4]. In contrast, hypoglycemia, a common acute complication of diabetes, is mainly regarded as being harmful for the brain. However, there is now increasing evidence that hypoglycemia can also have negative effects on the vasculature, similarly to hyperglycemia, and associations with myocardial infarction, angina and acute cerebrovascular events have been reported [5]. However, little is known on the mechanisms beyond these associations.

Interestingly, the present study and a similar one published on the same issue of *Diabetes Care* [6], have shown, through physiological studies, that moderate acute hypoglycemia can activate several pathways known to damage the vasculature. By performing hyperinsulinemic euglycemic and hypoglycemic clamps, it was possible to control for the known effects of insulin on the blood vessels, including anti-inflammatory and antiatherogenic properties, and therefore independently study the direct effect of acute reductions in glucose levels. Therefore, moderate hypoglycemia, lasting for 2 h, was associated with the activation of proinflammatory molecules and platelets, and with decreased fibrinolytic activity, both in individuals with T1D and healthy controls.

Although the study conditions were different from what can happen in daily life in people with T1D who are exposed to hypoglycemic episodes of variable duration and intensity, this study offers good evidence of an independent effect of low glucose levels on proinflammatory and atherogenic mechanisms and further strengthens the importance of avoiding hypoglycemia.

New mechanisms **The emerging role of NK cells in T1D**

Natural killer cells from children with type 1 diabetes have defects in NKG2D-dependent function and signaling

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Diabetes 2011;60:857–66.

Background: Natural killer (NK) cells from NOD mice have numeric and functional abnormalities, and restoration of NK cell function prevents autoimmune diabetes in NOD mice. The aim of this study was to assess the phenotype and function of NK cells in a large cohort of children with T1D.

Methods: Peripheral blood mononuclear blood cells were obtained from children with a T1D duration between 6 months and 2 years. NK cells were characterized by flow cytometry, enzyme-linked immunosorbent spot assays, and cytotoxicity assays. Signaling through the activating NK cell receptor, NKG2D, was assessed by immunoblotting and reverse-phase phosphoprotein lysate microarray.

Results: The number of NK cells in T1D subjects was reduced compared with age-matched, non-diabetic controls. NK cells from T1D subjects had diminished responses to interleukin (IL)-2 and IL-15. Analysis before and after IL-2 stimulation revealed that NK cells from T1D subjects failed to downregulate the NKG2D ligands, major histocompatibility complex class I-related chains A and B (MICA/B), upon activation. T1D NK cells also exhibited decreased NKG2D-dependent cytotoxicity and interferon- γ secretion. Finally, T1D NK cells showed clear defects in NKG2D-mediated activation of the phosphoinositide 3-kinase-AKT pathway.

Conclusions: This is the first study showing that T1D subjects have aberrant signaling through the NKG2D receptor and suggest that NK cell dysfunction contributes to the pathogenesis of T1D.

NK cells play a key role within the immune system, by directly killing cells infected by pathogens as well as by producing proinflammatory cytokines, such as interferon- γ and tumor necrosis factor- α [7]. NK cell can also act both as regulators and inducers of autoimmune diseases, and there is evidence indicating that their number and function are impaired in NOD mice [8]. The role of NK cells in the development of autoimmune diseases could be mediated through direct tissue destruction or indi-

rectly through the regulation of adaptive immune responses or the modification of antigen presenting cells [9].

The study by Qin et al. is the first in assessing NK cell number and function in a large cohort of children with T1D. Interestingly, they found that NK cell frequency was reduced by around 37% in children with T1D when compared to healthy controls. The decreased number of NK cells was associated with a reduced function, as indicated by impaired response to cytokines, such as IL-2/IL-15, which failed to downregulate the expression of the ligands MICA/B. These ligands normally act through a key NK cell receptor, the NKG2D, and in this study an intracellular signal transduction defect proximal to the NKG2D receptor was detected. Taken together, these findings indicated that significant defects in the number and function of NK cells are detectable in young people with T1D, and suggest that an immune-modifying therapy increasing NK cell number and function may be valuable in T1D.

New mechanisms **Blocking glucagon action to treat T1D**

Glucagon receptor knockout prevents insulin-deficient type 1 diabetes in mice

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Diabetes 2011;60:391–7.

Background: To determine the role of glucagon action in the metabolic phenotype of untreated insulin deficiency.

Methods: Clinical and metabolic parameters were compared between glucagon receptor-null ($Gcgr^{-/-}$) mice and wild-type ($Gcgr^{+/+}$) controls after equivalent destruction of β cells. A double dose of streptozotocin was used in this experiment to maximize β -cell destruction.

Results: $Gcgr^{+/+}$ mice became hyperglycemic, hyperketonemic, polyuric, and cachectic and had to be killed after 6 weeks. In contrast, none of these manifestations was apparent in $Gcgr^{-/-}$ mice, which underwent a comparable β -cell destruction. There was marked α -cell hyperplasia and hyperglucagonemia, but hepatic phosphorylated cAMP response element binding protein and phosphoenolpyruvate carboxykinase mRNA were profoundly reduced compared with $Gcgr^{+/+}$ mice with diabetes, confirming complete blockage of glucagon action. Fasting glucose levels and oral and intraperitoneal glucose tolerance tests were normal. Both fasting and non-fasting free fatty acid levels and non-fasting β -hydroxybutyrate levels were lower.

Conclusions: Blocking glucagon action prevents the deadly metabolic and clinical derangements of T1D in mice.

In the context of T1D, pancreatic β cells are extensively replaced by α cells and hyperglucagonemia has been suggested as a key player in the metabolic phenotype associated with insulin deficiency [10]. This has led to investigations based on glucagon suppression as a potential therapeutic strategy in T1D. The first attempt to block glucagon was made following the development of the glucagon suppressor somatostatin, which was able to prevent the acute metabolic derangements associated with lack of insulin [11]. Subsequently, leptin was proven to be another valuable glucagon antagonist, but given its additional metabolic effects, it was difficult to conclude whether suppression of glucagon per se was able to prevent the catabolic status associated with insulin deficiency [12].

In the present study, the authors further explored this issue using a different approach, based on blocking the action of glucagon. Two populations of mice were studied, both with diabetes induced by streptozotocin, but in one group the glucagon receptor was active, whereas in the other was not expressed. Interestingly, in contrast to the wild-type mice model, the glucagon-receptor null mice did not develop hyperglycemia or other typical manifestations of diabetes. Alpha cells were significantly hyperplastic in these mice, but glucagon action was confirmed to be completely blocked by the mark-

edly reduced levels of a gluconeogenic enzyme, such as phosphoenolpyruvate carboxylase mRNA and free fatty acids.

The findings of this study highlight the key role of glucagon in mediating the metabolic phenotype of insulin deficiency and call for further studies aiming at developing antagonists of glucagon action to be explored in humans as a potential approach to treat T1D.

New mechanisms

Looking for the cause of vitamin D deficiency in T1D

Enhanced excretion of vitamin D binding protein in type 1 diabetes: a role in vitamin D deficiency?

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Background: Vitamin D deficiency is a frequent finding in patients with diabetes, and it has been associated with diabetic nephropathy. The aim of this study was to assess whether urinary loss of vitamin D binding protein (VDBP) might contribute mechanistically to vitamin D deficiency.

Methods: Plasma and urine VDBP as well as serum concentrations of total calcium, parathyroid hormone, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D were compared between 115 subjects with T1D (age 14–40 years) and 55 age-matched healthy controls and also analyzed in relation to the presence of albuminuria.

Results: Urinary excretion of VDBP was significantly increased in subjects with T1D, compared with control subjects. Urinary VDBP excretion was independently associated with microalbuminuria ($p = 0.004$), HbA1c ($p = 0.010$), continuous glucose monitoring system average capillary glucose ($p = 0.047$), and serum 1,25-dihydroxyvitamin D concentrations ($p = 0.037$). Vitamin D deficiency or insufficiency was slightly more prevalent in diabetic subjects with albuminuria, coincident with the increase in urine VDBP excretion.

Conclusions: In subjects with T1D, and in particular in those with albuminuria, exaggerated urinary loss of VDBP could contribute to vitamin D deficiency.

Vitamin D deficiency has been reported in people with T1D and type 2 diabetes and has been suggested as a potential factor predisposing to the development of these diseases [13]. In addition, several recent studies also suggest a role of vitamin D deficiency in the pathogenesis of diabetic vascular complications, such as nephropathy and retinopathy [14, 15]. An important question to be answered is why people with diabetes present vitamin D deficiency.

Reduced intake/production could be one reason and defects in the enzymes/pathways implicated in the production of the activated form of vitamin D in the body could be another valid explanation. A third mechanism could be an increased loss, due for example to reduced levels of VDBP, which is the main binding protein of vitamin D metabolites. In humans, 88% of 25-OH vitamin D binds to VDBP, and this complex is filtered by the glomerulus and then reabsorbed in the proximal tubular cells through binding to megalin, a multiligand receptor expressed by several epithelial cells. Megalin is implicated in the renal reabsorption of several molecules and hormone, such as vitamin-binding proteins and albumin. In people with T1D and microalbuminuria, excretion of megalin is increased and this might lead to increased urinary losses of VDBP and vitamin D [16].

In the present study, excretion of VDBP and other components of the calcium metabolism were compared between subjects with and without T1D. Interestingly, VDBP excretion was increased in people with T1D, particularly in those with microalbuminuria, who also presented a slightly higher prevalence of vitamin D deficiency. Given the critical role of VDBP in delivering 25-OH-vitamin D to the renal epithelial cells, for reabsorption and subsequent activation to the active 1,25-OH vitamin D form, abnormal urinary loss of VDBP could represent a new mechanism explaining reduced vitamin D

in subjects with T1D and the potential missing link between decreased vitamin D levels and the reported increased risk of diabetic nephropathy.

New genes

RAGE: a link between genetic and environmental factors in T1D

Receptor for advanced glycation end-products (RAGE) provides a link between genetic susceptibility and environmental factors in type 1 diabetes

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Diabetologia 2011;54:1032–42.

Background: The aim of this study was to assess human genetic susceptibility conferred by the receptor for advanced glycation end-products (RAGE) in T1D and how this may interact with a Western environment.

Methods: The AGER gene was analyzed, using 13 tag SNPs, in 3,624 Finnish individuals from the FinnDiane study, followed by AGER associations with a high-risk HLA genotype (n = 546; HLA-DR3/DR4), matched in healthy newborn infants from the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) Study (n = 373) using allelic analysis. Islets and circulating RAGE were also studied in NODLt mice.

Results: The rs2070600 and rs17493811 AGER polymorphisms predicted increased risk of T1D, whereas the rs9469089 SNP was related to decreased risk, on a high-risk HLA background. Children from the DIPP study also showed a decline in circulating soluble RAGE levels at seroconversion to positivity for T1D-associated autoantibodies. Islet RAGE and circulating soluble RAGE levels in prediabetic NODLt mice decreased over time and were prevented by the AGE lowering therapy alagebrium chloride. Alagebrium chloride also decreased the incidence of autoimmune diabetes and restored islet RAGE levels.

Conclusion: These studies suggest that inherited AGER gene polymorphisms may confer susceptibility to environmental insults. Declining circulating levels of soluble RAGE, before the development of overt diabetes, may also be predictive of clinical disease in children with high- to medium-risk HLA II backgrounds.

Advanced glycation end products (AGEs) have for long time been known to be implicated in the pathogenesis of diabetic complications. Interestingly, there is also evidence for their role in pancreatic β -cell dysfunction [17]. AGEs are produced as a result of hyperglycemia, oxidative stress and ageing. In addition, diet can be another source of these products. AGEs effects are mainly mediated through RAGE, a multiligand receptor encoded by the AGER gene [18]. The activation of RAGE by AGE ligands is followed by inflammatory responses and an increased production of reactive oxidant species, activation of monocytes, induction of a procoagulant status and inhibition of nitric oxide activity.

In the present study, Forbes et al. report an interesting association between three polymorphisms in the AGER gene and susceptibility to T1D. In addition, another key finding of the study was that circulating levels of soluble RAGE declined at seroconversion for T1D autoantibodies and predicted T1D risk. Furthermore, an excess of environmental factors, such as AGEs, influenced circulating levels of RAGE as well as the expression of intraislet RAGE, which in turn could contribute to dysfunction of insulin secretion. In a mouse model, treatment with an AGE inhibitor, alagebrium chloride, significantly increased the expression of soluble RAGE and reduced the incidence of autoimmune diabetes. Overall, these findings indicated that AGER gene polymorphisms can contribute to the susceptibility to T1D, and RAGE represents an example of convergence of genetic and environmental influences, which can therefore modulate its expression and circulating levels and in this way contribute to the development of T1D.

Inherited variation in vitamin D genes is associated with predisposition to autoimmune disease type 1 diabetes

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Diabetes 2011;60:1624–31.

Background: Vitamin D deficiency is commonly reported in both children and adults and growing evidence indicates it is associated with many extraskeletal chronic disorders, including autoimmune diseases, such as T1D and multiple sclerosis.

Methods: 25-Hydroxyvitamin D (25(OH)D) was assessed in samples collected from 720 T1D cases and 2,610 controls and single nucleotide polymorphisms from seven vitamin D metabolism genes were genotyped in 8,517 cases, 10,438 controls, and 1,933 family samples.

Results: T1D patients had lower circulating 25(OH)D levels than similarly aged subjects from the British population. Only 4.3 and 18.6% of T1D patients reached optimal levels (≥ 75 nmol/l) of 25(OH)D for bone health in the winter and summer, respectively. Previous reported associations between four vitamin D metabolism genes (GC, DHCR7, CYP2R1, and CYP24A1) and 25(OH)D were replicated in control subjects. In addition to the previously reported association between T1D and CYP27B1 ($p = 1.4 \times 10^{-4}$), T1D was also consistently associated with DHCR7 ($p = 1.2 \times 10^{-3}$) and CYP2R1 ($p = 3.0 \times 10^{-3}$).

Conclusion: Circulating levels of 25(OH)D in children and adolescents with T1D vary seasonally and are under the same genetic control as in the general population but are much lower. Three key 25(OH)D metabolism genes were associated with T1D risk, indicating a genetic etiological role for vitamin D deficiency in T1D.

There is growing interest in the link between vitamin D deficiency/insufficiency and increased risk of autoimmune diseases, including T1D. The association between vitamin D and T1D is suggested by the observation of a higher incidence of the disease at higher latitudes and by the typical seasonal pattern of T1D, with a peak incidence during winter and the lowest incidence during summer.

Circulating 25-OH vitamin D concentrations are influenced by sun exposure and diet, but there is also evidence supporting a key role of genetic factors, with heritability estimates between 29 and 40% [19]. A recent genome-wide association study (GWAS) has identified 4 genetic variants associated with 25-OH vitamin D: GC, DHCR7, CYP24A1, CYP2R1. These variants were near genes involved in cholesterol synthesis, hydroxylation, and vitamin D transport [20].

In the current study the authors assessed the relationship between vitamin D and T1D through a comparison of vitamin D levels between subjects with T1D and controls from the general population, and by assessing the relationship of vitamin D genetic variants with T1D.

First of all, this study confirmed the already known seasonality of variation in the incidence of T1D. In addition, it was confirmed that there is a seasonal variation in vitamin D levels with the highest incidence of cases of deficiency/insufficiency during winter and spring. In addition, suboptimal and deficient levels of vitamin D were definitely more common among subjects with T1D than controls. Interestingly, the authors were able to confirm the association of previously reported GWAS loci with vitamin D. Even more interesting was the finding of a consistent association of three vitamin D loci, CYP27B1, DHCR7 and CYP2R1, with T1D.

What is the link between vitamin D and T1D? It has been suggested that decreased vitamin D levels are associated with reduced T-cell activation and proliferation, and therefore T-cells' hyporesponsiveness could represent the missing link between vitamin D deficiency and risk of developing T1D [21]. The findings of the present study support the potential role of vitamin D supplementation as a preventive strategy for T1D. Further studies should assess whether vitamin D supplementation can restore normal circulating levels in patients with T1D and affects the immunophenotype associated with T1D.

A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk

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Nature 2010;467:460–4.

Background: Combined analyses of gene networks and DNA sequence variation can provide new insights into the etiology of common diseases that may not be apparent from genome-wide association (GWA) studies alone.

Methods: This study used an integrated genome-wide approach applied across seven rat tissues to identify gene networks and the loci underlying their regulation. GWA was also performed in monocytes derived from two human cohorts.

Results: The study was able to identify an interferon regulatory factor 7 (IRF7)-driven inflammatory network (IDIN) enriched for viral response genes, which represents a molecular biomarker for macrophages and which was regulated in multiple tissues by a locus on rat chromosome 15q25. Epstein-Barr virus induced gene 2 (Ebi2, also known as Gpr183), which lies at this locus and controls B-lymphocyte migration, is expressed in macrophages and regulates the IDIN. The human locus on chromosome 13q32 controlled the human equivalent of the IDIN. IDIN genes were more likely to associate with susceptibility to T1D than randomly selected immune response genes ($p = 8.85 \times 10^{-6}$). The human locus controlling the IDIN was associated with the risk of T1D at a single nucleotide polymorphism rs9585056 ($p = 7.0 \times 10^{-10}$; odds ratio 1.15), which was one of five single nucleotide polymorphisms in this region associated with EBI2 expression.

Conclusions: These data implicate IRF7 network genes and their regulatory locus in the pathogenesis of T1D.

Genome-wide association studies (GWAS) have identified many genetic variants associated with complex diseases, such as T1D. However, the data so far generated need to be translated into biological function by correlating DNA variations with gene expression in order to identify gene networks involved in the pathogenesis of diseases.

In the present study a new approach is presented based on the combination of analyses of gene networks and DNA sequence variation. GWA data were generated from seven rat tissues and led to the identification of an interferon regulatory factor 7 (IRF7)-driven inflammatory network (IDIN) enriched for viral response genes, which represents a molecular biomarker for macrophages, and which was regulated in multiple tissues by a locus on rat chromosome 15q25. In order to translate these findings to humans, GWA data from monocytes obtained from around 2,000 individuals were assessed and a human locus localized on chromosome 13q32 was found to control the human equivalent of the IDIN. Interestingly, the human locus controlling the IDIN was associated with risk of T1D. This complex approach appears to be a new valid strategy to identify the molecular basis of common diseases.

Effectiveness of sensor-augmented insulin-pump therapy in type 1 diabetes

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N Engl J Med 2010;363:311–20.

Background: Recently developed technologies for the treatment of T1D include a variety of pumps and pumps with glucose sensors.

Methods: In this 1-year, multicenter, randomized, controlled trial, the efficacy of sensor-augmented pump therapy (pump therapy) was compared with that of a regimen of multiple daily insulin injections (injection therapy) in 485 patients (329 adults and 156 children) with inadequately controlled T1D. The primary end-point was the change from the baseline glycated hemoglobin level.

Results: At 1 year, the baseline mean glycated hemoglobin level (8.3% in the two study groups) had decreased to 7.5% in the pump-therapy group, as compared with 8.1% in the injection-therapy group ($p < 0.001$). The proportion of patients who reached the HbA1c target ($<7\%$) was greater in the pump-therapy group than in the injection-therapy group. The rate of severe hypoglycemia in the pump-therapy group (13.31 cases per 100 person-years) did not differ significantly from that in the injection-therapy group (13.48 per 100 person-years, $p = 0.58$). There was no significant weight gain in either group.

Conclusions: In both adults and children with inadequately controlled T1D, sensor-augmented pump therapy resulted in significant improvement in HbA1c levels and higher proportion of patients with HbA1c in the target levels.

Continuous subcutaneous insulin infusion and continuous glucose monitoring represent two important systems, which have contributed to improving the management of patients with T1D. Several recent studies have shown the beneficial effect of each of these two systems considered separately in ameliorating glycemic control. The study reported by Bergenstal et al. marks a further important step in the management of T1D, through the implementation of a sensor-augmented pump therapy, which integrates two technologies: pumps and continuous glucose monitoring, and allows monitoring treatment and response through an internet-based software. The Sensor-Augmented Pump Therapy for A1C reduction (STAR)-3 was a randomized controlled trial conducted in 30 diabetes centers in the USA and Canada with the aim of comparing the effect of a 1-year use of a sensor-augmented pump therapy versus a multiple injection regimen with insulin analogues on HbA1c levels in 485 adults and children with T1D with inadequately glycemic control and who were pump-naïve.

In the adult group, the sensor-augmented pump therapy was associated with a 1% decline in HbA1c after 1 year compared to the multiple injection regimen. A similar positive result was found in children, although similarly to previous studies, the effect was smaller (0.5%). Interestingly, the percentage of adults and children reaching HbA1c within recommended targets in the pump therapy group was higher than in the control group, without any significant increase in hypoglycemic episodes and weight gain. These findings are encouraging and promising, given that reaching a good glycemic control and containing at the same time the risk for hypoglycemia and weight gain still represents a big challenge in the management of diabetes. The study did not have a control group to test the effect of pump therapy alone versus sensor-augmented pump therapy, which could have allowed to understand whether the effect is definitely due to the combination of the two devices. Therefore, the question as to whether the observed treatment effect is due to the pump, the sensor or the combination of the two remains open and requires further investigations.

It is now important to translate these results in daily clinical practice, which differs from the clinical trial setting, where more frequent contact with study team have a considerably effect on results, and test whether these new strategy could add value to the lives of all patients of T1D.

Cause-specific mortality trends in a large population-based cohort with long-standing childhood-onset type 1 diabetes

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Diabetes 2010;59:3216–22.

Background: There is scant information on the primary causes of mortality in T1D responsible for the excess mortality seen in this population.

Methods: The Allegheny County Childhood-Onset Type 1 Diabetes Registry (n = 1,075, age at diagnosis <18 years) with diagnosis from 1965 to 1979 was used to assess cause-specific mortality. Cause of death was determined by a mortality classification committee of at least three physician epidemiologists, based on the death certificate and additional records surrounding the death.

Results: Vital status for 1,043 (97%) participants was ascertained as of 1 January 2008, revealing 279 (26.0%) deaths. During the first 10 years after diagnosis, the leading cause of death was acute diabetes complications (73.6%), while during the next 10 years, deaths were nearly evenly attributed to acute (15%), cardiovascular (22%), renal (20%), or infectious (18%) causes. After 20 years' duration, chronic diabetes complications accounted for >70% of all deaths, with cardiovascular disease as the leading cause of death (40%). Women ($p < 0.05$) and African-Americans ($p < 0.001$) have significantly higher diabetes-related mortality rates than men and Caucasians, respectively. Standardized mortality ratios for non-diabetes-related causes do not significantly differ from the general population.

Conclusions: The excess mortality seen in T1D is almost entirely related to diabetes and its comorbidities but varies by duration of diabetes and is higher in women and African-Americans.

There is a large body of evidence indicating that people with T1D have an increased age-adjusted mortality risk compared with the general population [22], although there are limited data based on a long-term follow-up of young people diagnosed with T1D during childhood.

Secrest et al. reported on cause-specific mortality in a large cohort with childhood-onset T1D in the USA. The study population was represented by the Allegheny County Type 1 Diabetes Registry, which recruited 1,075 young people, diagnosed between 1965 and 1979, and followed them for over 30 years. Two other interesting papers have been published in 2010–2011 on this cohort and have extensively assessed overall mortality and cause-specific mortality in T1D [23, 24].

Vital status was ascertained for 97% of the cohort and led to the identification of 279 deaths (26%). Total mortality declined over time, by about 15%, from 1965–1969 to 1975–1979. In particular, mortality for acute complications declined by 57%, mortality for renal disease by 38% and mortality for cardiovascular disease by 30%. This declining trend with date of diagnosis is positive and encouraging, and likely reflects improvements in diagnosis and management of diabetes over time. Less encouraging was the finding that overall mortality rates among the T1D population were significantly higher than in the non-diabetic population: being 13 times higher for CVD, 104 times higher for renal disease and 41 times higher for infections. In contrast, there was no significant difference in standardized mortality rates for non-diabetes-related cause. Cause-specific mortality differed with diabetes duration. Acute complications, mainly diabetic ketoacidosis, represented the main causes of death during the first 10 years from diabetes diagnosis, being responsible for more than 70% of all cases. In contrast, after 10 years of duration, acute complications determined less than 20% of all deaths, whereas chronic complications such as renal disease and cardiovascular disease became dominant. Renal disease contributed to 50% of cases of deaths after 10 years and CVD became the dominant cause after 20 years (60% of all deaths). Within the T1D population, mortality for diabetes-related causes was higher among African-American and females, and this could be related, at least for African-Americans, to socioeconomic issues, such as less access to care, although this is not a likely explanation for the higher mortality in females.

The key message beyond the current study findings is that there is a great need of implementing better preventing and therapeutic strategies for diabetic complications, both acute and chronic, in order to improve the long-term prognosis of patients with T1D.

Low levels of asymmetric dimethylarginine in children with diabetes mellitus type 1 compared with healthy children

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J Pediatr 2011;158:602–606 e1.

Background: High levels of asymmetric dimethylarginine (ADMA) have been associated with an increased risk for vasculopathy in adults but have also been detected in healthy young children. The aim of the present study was to assess ADMA concentrations in pediatric patients with T1D and in healthy controls.

Methods: In this cross-sectional study, ADMA was measured in 85 pediatric patients with T1D and 89 age- and sex-matched healthy controls.

Results: ADMA concentrations were significantly lower in patients with T1D and were inversely correlated with HbA1c concentrations.

Conclusions: Besides its vasoprotective function, nitric oxide itself may exert oxidative stress by generating free radicals. In this context, ADMA would protect the system from nitric oxide overproduction and perpetuation of oxidative stress. Thus, low ADMA concentrations in children with DM1 may be an indicator of impaired protection against oxidative stress.

Asymmetric dimethylarginine in young people with type 1 diabetes: a paradoxical association with HbA1c

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Diabet Med 2011;28:685–91.

Background: Asymmetric dimethylarginine (ADMA) is an independent risk factor for cardiovascular disease and its concentrations are increased in several diseases, including diabetes. The aim of this study was to perform a longitudinal evaluation of plasma ADMA and of its determinants in young people with childhood-onset T1D.

Methods: ADMA was measured in 1,018 longitudinal blood samples collected from 330 young people with T1D followed in the Oxford Regional Prospective Study. Additional data concerning annual assessments of HbA1c, height, weight, insulin dose and three early morning urine samples for measurement of the albumin/creatinine ratio were available.

Results: ADMA levels were significantly higher in males than in females (0.477 ± 0.090 vs. 0.460 ± 0.089 $\mu\text{mol/l}$, $p = 0.002$) and declined with chronological age (estimate $\pm$ SE: -0.0106 ± 0.0008 , $p < 0.001$). A significant inverse association was detected between ADMA and HbA1c (0.0113 ± 0.001 , $p < 0.001$). ADMA levels were lower in subjects developing microalbuminuria than in subjects with normoalbuminuria, but this difference disappeared after adjusting for HbA1c.

Conclusions: In this longitudinal study, ADMA concentrations decreased with age and were significantly higher in males and lower in subjects developing microalbuminuria. These associations were largely explained by a paradoxical negative association between HbA1c and ADMA.

ADMA, an endogenous inhibitor of nitric oxide synthase (NOS), is a naturally occurring arginine analogue, produced during posttranslational methylation of arginine residues in nuclear proteins and subsequently released by proteolysis [25]. ADMA has emerged as an important risk factor for cardiovascular disease (CVD). Important associations between increased plasma ADMA levels and poor clinical outcomes have emerged in several clinical conditions, including chronic renal disease, hypertension, dyslipidemia, insulin resistance, diabetes mellitus, and liver disease [26]. Up to now, few data are available for the pediatric population. Interestingly, these two studies point towards a revision of

the concept of ADMA being increased in the context of diabetes. In fact, in the paper by Huemer et al., ADMA levels were decreased in children with T1D compared to matched controls. In the second paper, ADMA was decreased in young people with T1D and microalbuminuria compared with those without this complication. Even more interesting was the finding of a negative association between HbA1c and ADMA in both studies. A similar relationship had previously being reported in a small study performed in adults with type 2 diabetes, whereas other studies have found no association or positive relationships with glycemic control. So, why is an emerging CVD risk factor such as ADMA reduced in youth with diabetes and inversely related to long-term glycemic control?

Reduced plasma ADMA levels might be due to hyperfiltration and increased renal clearance, which are common findings in adolescents with T1D. However, hyperglycemia might also downregulate mechanisms implicated in the synthesis/production of ADMA or stimulate the metabolism/clearance of ADMA in other tissues/organs, such as the liver.

Are reduced ADMA levels beneficial or detrimental in youth with T1D? At the moment it is difficult to give a definitive answer to this question. Increased ADMA levels have been claimed to be detrimental due to the associated reduced nitric oxide levels. However, given that nitric oxide per se can induce oxidative stress, it may be possible that the decreased ADMA levels in people with T1D might have a negative effect on the vascular milieu as this may further facilitate NOS overexpression and increased superoxide production and consequent CVD.

New hormones Beyond the classical action of erythropoietin

Erythropoietin protects against diabetes through direct effects on pancreatic β cells

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J Exp Med 2010;207:2831–42.

Background: Evidence has suggested that erythropoietin (EPO) exerts cytoprotective effects on non-erythroid cells. The aim of this study was to assess the influence of EPO on pancreatic β cells and diabetes.

Methods: Streptozotocin-induced and db/db mouse models of T1D and type 2 diabetes were used and recombinant EPO was administered.

Results: EPO exerted antiapoptotic, proliferative, anti-inflammatory, and angiogenic effects within the pancreatic islets. Using β -cell-specific EPO receptor and JAK2 knockout mice, it was found that these effects derived from direct biological effects of EPO on β cells and that JAK2 is an essential intracellular mediator.

Conclusions: Promotion of EPO signaling in β cells may be a novel therapeutic strategy for diabetes prevention and treatment.

Loss of β cells is a common feature of both T1D and type 2 diabetes, and leads to lack of insulin and severe metabolic derangements. Therefore, the preservation of β cells represents an attractive target for strategies aiming at preventing and treating both types of diabetes.

There is evidence suggesting that EPO can exert functions other than regulating erythropoiesis, and its receptor is present in non-erythroid tissues, such as the brain, kidney and pancreas. The observation that the EPO receptor is present in the pancreas, where it prevents cytokine-induced cell death, together with the higher incidence of anemia and EPO deficiency in people with diabetes and the hypoglycemic effect of EPO treatment previously reported, led the authors to develop their hypothesis.

The authors conducted an elegant experimental study using mice models of T1D and type 2 diabetes, and they clearly showed that this hormone has beneficial effect on β cells, mainly characterized by promotion of β -cell survival and growth rather than β -cell function. In the pancreatic β cells, EPO binds to its receptor, which belongs to the cytokine class I receptor superfamily, and its downstream effects are mainly mediated through the JAK2 kinase. These lead to significant effects, such as pre-

vention of apoptosis together with stimulation of proliferation and angiogenesis of the β cells. Therefore, in the present study, EPO emerged as a preventive therapeutic strategy against the development of diabetes in mice models of T1D and type 2 diabetes and these findings shed a new light and hope for the prevention and treatment of diabetes.

Mechanism of the year

An emerging insulin-sensitive cell: the podocyte

Insulin signaling to the glomerular podocyte is critical for normal kidney function

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Cell Metab 2010;12:329–40.

Background: Diabetic nephropathy is characterized by albuminuria and abnormal glomerular function and is microvascular complication of diabetes, implying a primary defect in the endothelium. The aim of this study was to determine whether insulin signaling in podocytes affects glomerular function *in vivo*.

Methods: For the present study, two podocyte-specific insulin receptor knockout mice (podIRKO) models were generated.

Results: These animals developed significant albuminuria together with histological features that recapitulate diabetic nephropathy, but in a normoglycemic environment. Examination of ‘normal’ insulin-responsive podocytes *in vivo* and *in vitro* demonstrates that insulin signals through the MAPK and PI3K pathways via the insulin receptor and directly remodels the actin cytoskeleton of this cell.

Conclusions: This study reveals the critical importance of podocyte insulin sensitivity for kidney function.

Diabetic nephropathy is a common complication of diabetes with a complex pathogenesis. Several lines of evidence have shown that, in patients with diabetes, insulin resistance correlates with the severity and onset of albuminuria, suggesting that insulin resistance per se may cause albuminuria [27].

In the present study, Welsh et al. have shown that a podocyte-specific absence of the insulin receptor leads to a glomerular lesion resembling that associated with diabetic nephropathy, but in the absence of hyperglycemia. Podocytes are highly specialized epithelial cells, interconnected by foot processes, which delimit the slit diaphragm, the main size-selective barrier in the glomerulus. The key role of the podocytes in the pathogenesis of renal diseases is supported by the strong association between podocyte-specific mutations and loss of albumin in the urine. Previous work from the same group showed that podocytes are insulin-sensitive cells able to absorb glucose [28].

The present study adds a further piece to the ‘podocyte story’, by showing that insulin sensitivity is a key element in maintaining the integrity of the podocytes. In mice with deletion of the insulin receptor albuminuria developed and this was associated with morphological renal alterations, characterized by effacement of the podocytes foot processes, apoptosis, thickening of the glomerular basement membrane and glomerulosclerosis. These changes in the podocytes are similar to those occurring in the context of diabetes, where they have been related to the degree of albumin excretion and decline in glomerular filtration rate. Interestingly, there was a significant variation in the severity of the renal phenotype of the mutant mice, suggesting that diabetic nephropathy might not develop in all cases bearing a mutant insulin receptor.

Insulin was shown to induce remodeling of the podocyte cytoskeleton through activation of the MAPK and PI3K pathways and the final effect was the retraction of cellular processes and increased permeability.

After this new fascinating study, the ‘podocyte story’ is not completely revealed yet. In fact, some findings derived from the present study, such as the development of non-specific renal changes as well as the lack of some specific kidney changes generally associated with diabetes, such as renal

hypertrophy and nodular glomerulosclerosis, in the mice lacking the insulin receptor suggest that there are other mechanisms still to be discovered. However, an important message of the present study is that renal insulin resistance may be an important player in the pathogenesis of diabetic kidney disease and might therefore represent a target for future therapies aiming at preventing diabetic nephropathy.

New paradigms

Enteroviruses accelerate progression from autoimmunity to diabetes

Enterovirus infection and progression from islet autoimmunity to type 1 diabetes: the Diabetes and Autoimmunity Study in the Young (DAISY)

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Diabetes 2010;59:3174–80.

Background: To assess whether enterovirus infections predict progression to T1D in genetically predisposed children repeatedly positive for islet autoantibodies.

Methods: The Diabetes and Autoimmunity Study in the Young (DAISY) has followed 2,365 genetically predisposed children for islet autoimmunity and T1D since 1993. Venous blood and rectal swabs were collected every 3–6 months after seroconversion for islet autoantibodies until diagnosis of diabetes. Enteroviral RNA in serum or rectal swabs was detected using reverse transcriptase PCR.

Results: Of 140 children who seroconverted to repeated positivity for islet autoantibodies at a median age of 4.0 years, 50 progressed to T1D during a median follow-up of 4.2 years. The risk of progression to clinical T1D in the sample interval following detection of enteroviral RNA in serum (3 diabetes cases diagnosed among 17 intervals) was significantly increased compared with that in intervals following a negative serum enteroviral RNA test (33 cases diagnosed among 1,064 intervals; hazard ratio 7.02 (95% CI 1.95–25.3) after adjusting for number of autoantibodies). Enteroviral RNA in rectal swabs was not predictive of progression to type 1 diabetes.

Conclusion: These observations suggest that progression from islet autoimmunity to T1D may increase after an enterovirus infection characterized by the presence of viral RNA in blood.

Enteroviruses, especially Coxsackie B virus, have been the most studied environmental factors in the context of T1D. Enteroviruses have been detected in the blood, gut, pancreas and stool of patients with T1D. A recent meta-analysis published in the BMJ in 2010 reported a significant association of enterovirus infections, detected with molecular methods, and autoimmunity and T1D [29].

The mechanisms by which enteroviruses are linked to diabetes are complex and probably involve an interplay between the virus, the immune system, the pancreatic β cells and the genotype of the patient [30]. However, animal studies have suggested that viruses are probably not able to initiate the autoimmune process, but more likely they accelerate an already started process. Following this hypothesis, the present study assessed, in the large DAISY cohort of children with increased genetic susceptibility to T1D, whether in those developing persistence positivity for autoantibodies, the presence of enteroviral RNA in the serum or rectal swabs predicted the progression to T1D. Of 140 children who seroconverted, 50 developed T1D; detection of serum enteroviral RNA was associated with a faster progression to T1D. The same was not true for positivity of rectal swab viral RNA. Although based on a small sample size, these results are of interest, as not only do they confirm a role of enteroviruses as environmental triggers for T1D, but represent the first line of evidence of the specific checkpoint where they act: that is, they increase the progression from autoimmunity to overt T1D. These results confirm findings from animal studies and indicate an important target for retarding progression to T1D.

Clinical and experimental pancreatic islet transplantation to striated muscle: establishment of a vascular system similar to that in native islets

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Diabetes 2010;59:2569–78.

Background: Transplantation of pancreatic islets into the liver is associated with poor long-term outcome and graft failure at least partly due to inadequate graft revascularization. The aim of this study was to assess whether striated muscle might be a potential angiogenic site for islet transplantation.

Methods: Islets were implanted into striated muscle and intraislet vascular density and blood flow were visualized with intravital and confocal microscopy in mice and by magnetic resonance imaging in three autotransplanted pancreatectomized patients. Mice were rendered neutropenic by repeated injections of Gr-1 antibody, and diabetes was induced by alloxan treatment.

Results: Islets transplanted to mouse muscle were revascularized with vessel densities and blood flow fully comparable with those of islets within intact pancreas. Initiation of islet revascularization at the muscular site was dependent on neutrophils, and the function of islets transplanted to muscle was proven by curing diabetic mice. The experimental data were confirmed in autotransplanted patients where high-resolution magnetic resonance imaging showed higher plasma volumes in islets engrafted in forearm muscle compared with adjacent muscle tissue.

Conclusions: This study presents a novel paradigm in islet transplantation based on the key role of the functionally restored intraislet blood perfusion following transplantation to striated muscle.

Islet transplantation has always been thought as the 'definitive cure for diabetes', and therefore intensive research has been focused on this field. However, many issues still limit this therapeutic approach and islet transplantation is far from being a widely applicable cure for T1D [31]. A major limitation is represented by lack of available cells to be transplanted. Another key issue is represented by the site where β cells are transplanted, which should resemble the native pancreatic islets as close as possible. The liver is the commonest site of engraftment, but it does not represent the optimal site for several reasons. In particular, one of the weaknesses of the liver in this context is a delayed and insufficient islet revascularization, which contrasts with the native islets, which are highly vascularized.

In the present study, Christoffersson et al. attempted to transplant islet cells in a striated muscle, the cremaster, in a mouse model. This resulted in a successful experiment, as demonstrated by the observation that within 2 weeks the vasculature within the islets was functionally and morphologically restored. Even more intriguing was the finding that the initiation of the revascularization in the muscle was strongly dependent on the neutrophils recruited to the implantation site, cells already known to be involved in muscle healing and regeneration.

Why is the revascularization process better in the muscle than in the liver? One suggestion is a different level of plasticity of endothelial cells in the muscle, and there is evidence indicating that muscular endothelium is programmed to proliferate during muscle overload and concomitant hypoxia [32].

The identification of this new promising site for β -cell engraftment together with the demonstration of the utility of a high-resolution magnetic resonance imaging for following the 'fate' of the transplanted β cells offer new hopes for improving the successful rate of islet transplantation.

A novel clinically relevant strategy to abrogate autoimmunity and regulate alloimmunity in NOD mice

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Diabetes 2010;59:2253–64.

Background: To investigate a new clinically relevant immunoregulatory strategy based on treatment with murine Thymoglobulin mATG Genzyme and CTLA4-Ig in NOD mice to prevent allo- and autoimmune activation using a stringent model of islet transplantation and diabetes reversal.

Methods: A new immunoregulatory protocol based on prolonged low-dose mATG plus CTLA4-Ig was assessed in allogeneic islet transplantation models as well as NOD mice with recent onset T1D.

Results: BALB/c islets transplanted into hyperglycemic NOD mice under prolonged mATG+CTLA4-Ig treatment showed a pronounced delay in allograft rejection compared with untreated mice (mean survival time: 54 vs. 8 days, $p < 0.0001$). Immunologic analysis of mice receiving transplants revealed a complete abrogation of autoimmune responses and severe downregulation of alloimmunity in response to treatment. The effect on autoimmunity was confirmed by 100% diabetes reversal in newly hyperglycemic NOD mice and 100% indefinite survival of syngeneic islet transplantation.

Conclusions: Prolonged mATG+CTLA4-Ig treatment was effective in regulating alloimmunity and to abrogate the autoimmune response in NOD mice in different settings. Therefore, prolonged mATG+CTLA4-Ig treatment represents a clinically relevant strategy to translate to humans with T1D.

In the previous paper from Christoffersson et al., the issue of unsuccessful revascularization of transplanted islets was investigated. However, another limiting factor for successful islet transplantation is that islet survival is affected by the development of alloimmunity and by the recurrence of autoimmunity. In the present study a new strategy based on combination of thymoglobulin, a widely used induction therapy for solid tumor and islet transplantation, and CTLA4-Ig, which mimic the inhibitory molecule expressed on activated T cells, was used in hyperglycemic mice transplanted with allogeneic islets and in mice undergoing a syngeneic transplant. The combination therapy was able to prolong the survival time of allogeneic islet cells, although final rejection was inevitable. Even more relevant was the effect of this combined therapy on autoimmunity. In fact, not only autoimmunity was completely abrogated in the context of allogeneic transplant, but the combination therapy was able to induce 100% diabetes reversal in newly hyperglycemic NOD mice and 100% indefinite survival of syngeneic islet transplantation.

The strength of the combination therapy is due to the synergic effect of the two drugs. Thymoglobulin depletes peripheral T cells and inhibits T-effector cell expansion, while saving T-regulatory cells. However, treatment with thymoglobulin is associated with the proliferation of residual or new lymphocytes. This is where CTLA4-Ig intervenes, by blocking such spontaneous re-emergence of lymphocytes. Given that both drugs are available for clinical use, the results of the present study are of utmost importance, suggesting that this combination therapy could be a novel treatment for T1D.

Non-invasive imaging of pancreatic islet inflammation in type 1A diabetes patients

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J Clin Invest 2011;121:442–5.

Background: A major impediment to advances in understanding, preventing, and curing T1D has been the inability to assess the disease initiate, progress, or regress, particularly during the occult phase. In this study, the development of a non-invasive method to visualize T1D at the target organ level in patients with active insulinitis is reported.

Methods: Islet inflammation, manifested by microvascular changes and monocyte/macrophage recruitment and activation was assessed using magnetic resonance imaging of magnetic nanoparticles (MRI-MNPs) in 10 patients with T1D and compared with pancreatic imaging in 12 controls.

Results: Imaging of infused ferumoxtran-10 nanoparticles permitted visualization of the pancreas and distinction of recent-onset diabetes patients from non-diabetic controls.

Conclusions: The observation that MNPs accumulate in the pancreas of T1D patients supports the role of this non-invasive imaging method in following T1D progression and monitoring the ability of immunomodulatory agents to contrast insulinitis.

T1D is a disease with a silent phase lasting for many years and characterized by the development and progression of insulinitis. Assessing this inflammatory subclinical stage of T1D is difficult as it requires pancreas biopsy or otherwise one can rely on the detection of circulating markers, such as autoantibodies or T cells, which however do not perfectly reflect the events occurring in the pancreas. Similar problems related to lack of optimal biomarkers are encountered for monitoring response to interventions with immunomodulators.

Starting from previous observations in mouse models of T1D [33], where the development of insulinitis is associated with characteristic inflammatory changes in the pancreatic microvasculature, mainly represented by vascular leakage, an MRI-magnetic nanoparticle (MRI-MNP) approach was developed, through the infusion of long-circulating phagotrophic nanoparticles, which extravasate from leaky vessels and are captured by infiltrating phagocytic cells. In mouse models this technique allowed the identification of typical characteristics of the disease and its aggressiveness and allowed the assessment of the response to immunomodulatory agents [34].

In the present study, the MRI-MNP was applied to 10 adult subjects with T1D and 12 controls. Ferumoxtran 10, acting as a T2-negative agent on MRI, was used as the MNP. All subjects underwent three MRI scans: a pre-MNP scan for baseline values, an immediate post-MNP, as an indicator of vascular volume and for pancreas volume estimates, and a delayed post-MNP after 48 h, to assess vascular leakage of MNPs and retention by phagocytic cells. Patients with T1D showed lower T2 signals after 48 h indicating higher retention of MNP. The variation in T2 signal before and 48 h post-MNP represents a measure of disease aggressiveness. Interestingly there was a wide variation in this index among T1D patients, likely reflecting heterogeneity in the insulinitis.

This study represents a first attempt to translate a new non-invasive biomarker of insulinitis from animal models to humans. Further evaluations of this technique will allow its implementation for identifying early stages of T1D. This could help in characterizing patients with more aggressive disease, who are particularly at risk. In addition, this MRI technique could be useful for monitoring, in a non-invasive manner, future interventions with immunomodulators.

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