Type 1 Diabetes: Clinical and Experimental

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Type 1 diabetes (T1D) is one of the most common chronic disorders of childhood and adolescence and its incidence is rising worldwide, with an annual increase over the last years of about 3–4% [1]. Based on recent estimates, between 2005 and 2010, there will be a 70% increase in the prevalence of T1D, with a doubling of new cases in children younger than 5 years [1]. These data are worrisome given that a diagnosis of T1D during childhood determines a longer exposure to the metabolic derangements of the disease when compared to adult-onset T1D, therefore increasing the burden of the disease [2]. In addition, an early onset of T1D is often associated with more acute presentations, such as diabetic ketoacidosis and admission to hospital, which increase T1D morbidity [3].

Many efforts are continuously made to better understand the pathogenesis of the disease, to identify subjects at increased risk of developing it, to improve daily management of T1D and to identify factors which could predict subjects particularly predisposed to the long-term vascular complications, and towards whom more aggressive and intensive interventions should be directed. The emergence of new and promising treatment options always creates optimism among clinicians and researchers, in particular when dealing with a chronic disease such as T1D and when the aim is to improve the future of our children. All the above-mentioned aspects are the focus of the articles chosen for the 2010 *Yearbook* chapter on T1D.

Mechanism of the year Another key role of the K_{ATP} channel

ATP-sensitive K^+ channel mediates the zinc switch-off signal for glucagon response during glucose deprivation

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Background: A recent hypothesis based on in vivo data from hypoglycemic rats proposes that glucagon secretion during hypoglycemia is triggered by a decrease in zinc co-secreted with insulin from β -cells, rather than the decrease in insulin itself. The aim of this study was to determine whether closure of the α -cell ATP-sensitive K^+ channel (K_{ATP} channel) is the mechanism through which the zinc switch-off signal triggers glucagon secretion during glucose deprivation.

Methods: Studies were performed using perfused isolated islets.

Results: The expected glucagon response to an endogenous insulin switch-off signal during glucose deprivation was observed in wild-type mouse islets. In experiments with streptozotocin-treated wild-type islets, a glucagon response to an exogenous zinc switch-off signal was observed during glucose deprivation. However, this glucagon response to the zinc switch-off signal during glucose deprivation was not seen in the presence of nifedipine, diazoxide, or tolbutamide or if K_{ATP} channel knockout mouse islets were used. All islets had intact glucagon responses to epinephrine.

Conclusions: This study shows that zinc co-release with insulin during glucose deprivation is a switch-off signal triggering glucagon secretion and that this zinc action is mediated through closure of K_{ATP} channels and consequent opening of calcium channels.

Glucagon release from pancreatic α -cells represents one of the main compensatory mechanisms stimulated by hypoglycemia. This response is often impaired, to a variable degree, in people with diabetes.

The 'intra-islet insulin hypothesis' is an intriguing hypothesis suggesting that glucagon secretion in conditions of glucose deprivation is mediated by the decreased intra-islet insulin secretion [4]. More recent studies have slightly modified this hypothesis proposing that other factors released by β -cells, such as γ -aminobutyric acid and zinc, together with insulin could suppress glucagon secretion when insulin levels are high and stimulate its release during hypoglycemia. In this context, zinc co-released with insulin has been shown to play an important role in several, although not all, experimental studies.

In the present study the authors mark a further step in the understanding of the intra-islet insulin hypothesis. Through a series of experiments in mice, they identified the closure of K_{ATP} channels as the key effector of the zinc switch-off signal for glucagon secretion during glucose deprivation. The first step of their studies was the confirmation that cessation of zinc co-release with insulin is a key mechanism to stimulate glucagon release during hypoglycemia. Then, through manipulation of the K_{ATP} receptor with drugs known to influence its activity, such as diazoxide, which keeps the channel opened, or tolbutamide, which keeps the channel closed, they found that the channel failed to respond to the switch-off zinc signal during glucose deprivation.

In a second model, where the SUR1 subunit of the K_{ATP} channel was knocked out, the impaired function of the channel led to lack of response to the switch-off zinc mechanism. A further step in their experiments was to block calcium channels with nifedipine, which therefore interfered with the downstream mechanisms following the closure of the K_{ATP} channels.

The present study is of utmost importance as it strengthens the hypothesis that a key issue for keeping a good glycemic control is the intercellular hormonal dialogue within the pancreatic islets, phenomenon which is lost in people with diabetes, for lack of β -cell insulin release. The results support the hypothesis that the association of high basal glucagon in T1D could at least in part be attributed to lack of insulin-bound zinc tonically suppressing α -cell function.

New paradigms 1

The early decline of β -cell glucose sensitivity

Progression to diabetes in relatives of type 1 diabetic patients: mechanisms and mode of onset

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Background: In this study the mode of onset of hyperglycemia and how insulin sensitivity and β -cell function contribute to the progression to T1D in relatives of patients with diabetes were assessed.

Methods: 328 islet cell autoantibody-positive, non-diabetic relatives from the observational arms of the Diabetes Prevention Trial-1 Study (median age 11 years [interquartile range (IQR): 8] underwent sequential OGTT at baseline, every 6 months, and 2.7 years later, when 115 subjects developed T1D. β -Cell glucose sensitivity (slope of the insulin-secretion/plasma glucose dose-response function) and insulin sensitivity were obtained by mathematical modeling of the OGTT glucose/C-peptide responses.

Results: At baseline, insulin sensitivity, fasting insulin secretion, and total post-glucose insulin output did not differ between progressors and non-progressors. In contrast, β -cell glucose sensitivity was significantly reduced in progressors (median 48 pmol/min/m²/mmol/l [IQR: 36] vs. 87 pmol/min/m²/mmol/l [IQR: 67]; $p < 0.0001$) and predicted incident diabetes ($p < 0.0001$) independently of gender, age, BMI, and clinical risk. Glucose sensitivity progressively declined over time with a significant fall 1.45 years before diagnosis. In contrast, 2-hour glucose levels did not significantly change until 0.78 years before diagnosis, when they started to rise rapidly (approx. 13 mmol $\cdot$ l⁻¹ $\cdot$ year⁻¹). During this anticipation phase, both insulin secretion and insulin sensitivity were essentially stable.

Conclusions: β -Cell glucose sensitivity is the earliest parameter to be impaired in relatives of patients with T1D and represents a strong predictor of diabetes progression. The time trajectories of plasma glucose

pattern over time is often biphasic, with a slow linear increase followed by a rapid increase, and are preceded by a further deterioration of β -cell glucose sensitivity.

A detailed understanding of the timeline of events characterizing the development of T1D in genetically predisposed individuals could help in defining when to intervene and with which strategy, in order to reduce the risk of progressing towards overt hyperglycemia, and hopefully prevent acute presentations of T1D. Although autoantibodies can be detected during the prodromic phase of T1D, they do not give any information on the time of onset of T1D, and up to now the natural history of pancreatic β -cells incompetence has not been well characterized.

The present study is unique in its design, including a large cohort of predisposed individuals with serial assessments of β -cell function and insulin sensitivity, in order to identify peculiar characteristics able to early distinguish, among predisposed individuals, those who will progress to T1D from non-progressors. This study sheds light on the role of ' β -cell glucose sensitivity', which represents the ability of β -cells to rapidly adapt to acute changes in plasma glucose. Interestingly, it was found that a decrease in this parameter is the earliest defect which characterizes progressors. A clear decline in β -cell glucose sensitivity was detected 1.4 years prior to diagnosis and over time represented the strongest predictor for diabetes. In contrast, no early alterations able to characterize predictors were found in insulin sensitivity and secretion. Insulin secretion showed an initial increase, reflecting an adaptation to raising glucose levels, and then decreased by only 20% at the time of diagnosis in progressors. In the majority of progressors plasma glucose showed a biphasic pattern, with an initial slow increase followed by a sudden rise around 0.7 years before diagnosis. At this time a further decline in glucose sensitivity was associated with a decline in insulin sensitivity and insulin secretion. In conclusion, this study indicates that in vivo β -cell glucose insensitivity is an early defect in T1D, which could be used as a valuable parameter to identify progressors to clinical diabetes.

New paradigms 2

Growing faster increases T1D risk

Height growth velocity, islet autoimmunity and type 1 diabetes development: the Diabetes Autoimmunity Study in the Young

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Background: The aim of the study was to assess a potential association of childhood size and growth rate with the development of islet autoimmunity (IA) and T1D.

Methods: The study population was represented by participants to the Diabetes Autoimmunity Study in the Young (DAISY), which, since 1993, has followed children at increased T1D risk, based on HLA-DR, -DQ genotype or family history, for the development of IA (defined as the presence of autoantibodies to insulin, GAD or protein tyrosine phosphatase islet antigen 2 twice in succession) and T1D. Height and weight were collected starting at age 2 years, and these parameters together with BMI and velocities of growth in height, weight and BMI were assessed in relation to the development of IA and T1D, which developed in 143 and 21, respectively, of the 1,714 DAISY children aged less than 11.5 years.

Results: Greater height growth velocity was associated with IA development (HR 1.63 [95% CI 1.31–2.05]) and even more strongly with the development of T1D (HR 3.34 [95% CI 1.73–6.42]) for a 1 SD difference in velocity.

Conclusions: In prepubertal children at increased genetic risk of T1D, greater height growth velocity may be involved in the progression from genetic susceptibility to autoimmunity and then to T1D.

A popular hypothesis maintains that the current childhood obesity epidemic is driving the increasing incidence and earlier age of T1D onset seen around the world. This study shows otherwise: an increased height velocity was associated with development of islet autoimmunity and progression to

T1D in prepubertal children genetically predisposed to T1D. In contrast, BMI and rapid weight gain were not associated with study outcomes.

These results raise the question as to whether increased height rate represents a direct stress factor for the β -cell, which would support the 'overload hypothesis' [5]. This hypothesis postulates that overload of the β -cells mediated by several mechanisms, including high growth rates, physical and psychological stress, insulin resistance, increases insulin demand and makes the β -cells more susceptible to autoimmune attacks and apoptosis [5].

However, it needs to be acknowledged that increased growth rate may be just an epiphenomenon or, to use the authors' words, 'a side effect' of the mechanisms driving the autoimmune disease process. It is possible that the *primum movens* is not the high height rate but a primary increase in insulin levels, probably genetically determined, which could lead to more rapid linear growth and higher demand for insulin from the β -cell.

The finding of a lack of association between IA/T1D and weight gain is in contrast with previous data in children [6]. However, differences in the characteristics of the study populations, such as age, presence or not of genetic susceptibility, might explain discordant findings across different studies. Interestingly, short stature was associated with autoimmunity but not with risk of progression to T1D. This is in contrast with previous findings showing an association between greater height and T1D and might reflect an adverse intrauterine environment associated later on with a higher growth velocity.

One potential explanation for the findings is that increased linear growth velocity, perhaps associated with higher levels of IGF-I, may result in greater insulin secretion and insulin resistance, which have also been shown to be associated with greater IGF-I levels. Further studies are required to clarify the link between increased growth velocity and T1D risk and clarify whether the 'overload hypothesis' can truly explain this association.

Important for clinical practice 1 **The metabolic memory wears off!**

Effect of prior intensive therapy in type 1 diabetes on 10-year progression of retinopathy in the DCCT/EDIC: comparison of adults and adolescents

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Background: To assess differences between adolescents and adults in the persistence of the benefits of intensive therapy 10 years after completion of the Diabetes Control and Complications Trial (DCCT).

Methods: Progression of retinopathy from DCCT closeout to year 10 of the Epidemiology of Diabetes Interventions and Complications (EDIC) was evaluated in 1,055 adults and 156 adolescents.

Results: During 10 years of follow-up, HbA1c was similar between previous DCCT intensive and conventional groups and between adolescents and adults. At EDIC year 10, progression of diabetic retinopathy was still slower in the intensively than in the conventionally treated adults (adjusted hazard reduction 56%, $p < 0.0001$), whereas in adolescents this beneficial effect had disappeared (32%, $p = 0.13$). The difference in the mean DCCT HbA1c between adolescents and adults (8.9 vs. 8.1%), mainly between the two intensively treated groups (8.1 vs. 7.2%), explained 79% of the observed differences between adults and adolescents in the metabolic effect on retinopathy progression.

Conclusions: Glycemic control during the DCCT study is a key player in the long-term complication risk. The waning of the metabolic memory in the adolescent cohort at year 10 of the EDIC strongly highlights the importance of establishing a strict glycemic control as early as possible and of maintaining it over time.

The DCCT and its observational follow-up study, EDIC, represent landmark studies in the field of T1D and its vascular complications. The DCCT undoubtedly showed that complication risk significantly

decreases with strict glycemic control both in adults and in adolescents [7]. The EDIC study raised the important concept of 'metabolic memory'; in other words, although after the end of the DCCT HbA1c levels became comparable between the intensively and conventionally treated groups, patients belonging to the first group still kept an advantage from prior better HbA1c values [8].

This recent paper from White and colleagues raises the important point as to whether the benefit of a strict glycemic control during the DCCT wears off in the long run, particularly with regard to retinopathy. The most intriguing finding of the study was the emergence of differences in the persistence of the metabolic memory between the DCCT adolescent and adult cohorts. Although, at year 10 of EDIC there was a decrease in the 3-step progression risk in the intensively treated adult group when compared with year 4, the advantage of the previous intensively treated patients still persisted and was still significant. In contrast, in the adolescent cohort, retinopathy progression at year 10 of the EDIC did not differ between the previous intensively and conventionally treated groups, thus indicating loss of the metabolic memory. Interestingly, 79% of the difference in the metabolic effect between adults and adolescents after 10 years from the end of the DCCT was due to the difference in the mean HbA1c levels during the DCCT between the two cohorts. This 1% difference, which did not seem to play a major role during the DCCT and early EDIC years with regard to the outcomes, emerges as a major player in the long run.

Although at the moment these results relate only to retinopathy, this is a key message, which once again strengthens the importance of establishing a good glycemic control as soon as possible in patients with T1D. Although youths with T1D rarely present advanced stages of complications, there is evidence that their pathogenesis starts soon after diagnosis and therefore preventive and therapeutic strategies should be implemented soon after diagnosis [9].

Important for clinical practice 2 **Predicting T1D risk in the general population**

Predictive characteristics of diabetes-associated autoantibodies among children with HLA-conferred disease susceptibility in the general population

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Background: The aim of this study was to assess the predictive performance of islet cell autoantibodies (ICAs) in combination with autoantibodies against insulin (IAAs), autoantibodies against GAD, and/or islet antigen 2 for T1D in children with HLA-defined disease predisposition recruited from the general population.

Methods: The study population was made of 7,410 children, who were observed from birth (median 9.2 years) for β -cell autoimmunity and T1D. If a child developed ICA positivity or diabetes, the three other antibodies were measured in all samples available from that individual. Persistent autoantibody positivity was defined as continued positivity in at least two sequential samples including the last available sample.

Results: Pre-diabetic ICA positivity was observed in 1,173 subjects (15.8%), 155 of whom developed T1D. 86% of 180 progressors (median age at diagnosis 5.0 years) were identified with ICA screening. Positivity for four antibodies was associated with the highest disease sensitivity (54.4%), negative predictive values (98.3%) and the lowest negative likelihood ratio (0.5). Combining persistent ICA and IAA positivity resulted in the highest positive predictive value (91.7%), positive likelihood ratio (441.8), cumulative disease risk (100%), and specificity (100%). Young age at seroconversion, high ICA level, multipositivity, and persistent positivity for IAA were significant risk markers for T1D.

Conclusion: The combination of HLA and autoantibody screening in the general population resulted in disease risks that are likely to be as high as those reported among autoantibody-positive siblings of children with T1D.

Diabetes-associated autoantibodies, which are detectable during the preclinical phase of diabetes, are known to predict the development of T1D in siblings [10]. In contrast, their predictive value in the general population is unknown. This study is unique as it is based on a large population of 7,410 children from a country with the highest risk of T1D in the world and who were observed from birth for β -cell autoimmunity and diabetes. The main inclusion criterion was carrying a high or moderate HLD haplotype for T1D susceptibility. During follow-up visits, blood samples were collected for measurement of ICAs, which were selected as the primary screening tool for β -cell autoimmunity. If these autoantibodies were found in two consecutive samples, the other autoantibodies, IA-2A, GADA and IAA, were assessed.

ICAs screening allowed to identify 86% of progressors, whereas this prediction was better when IAA were also assessed (97%). The combination of all four antibodies led to the highest disease sensitivity (54.4%), negative predictive value (98.3%) and the lowest likelihood ratio (0.5). The highest positive predictive value was obtained with the combination of ICA and IAA, reaching also the highest specificity and cumulative disease risk. The addition of GADA to ICA did not appear to improve prediction.

Similarly to what is already known for first-degree relatives of children with T1D, a younger age at seroconversion, high ICAs levels and multipositivity for autoantibodies were associated with a higher risk of diabetes.

Taken together, the findings of the present study suggest that HLA-genotyping together with regular assessment of autoantibody will represent an important tool to identify subjects at risk for T1D in the general population, once preventive programs are developed. Similar screening strategies will be of utmost importance, particularly in countries with a high incidence of T1D.

New genes

Genome-wide association studies finally approach HbA1c

A genome-wide association study identifies a novel major locus for glycemic control in type 1 diabetes, as measured by both A1C and glucose

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Background: Although glycemic control is a key risk factor for diabetic complications, there are no data on its potential genetic determinants in patients with T1D. The aim of this study was to identify genetic loci associated with glycemia using longitudinal repeated measures of HbA1c from the Diabetes Control and Complications Trial (DCCT).

Methods: A genome-wide association study was performed using the mean of quarterly HbA1c values measured over 6.5 years, separately in the conventional ($n = 667$) and intensive ($n = 637$) treatment groups of the DCCT. At loci of interest, linear mixed models were used to take advantage of all the repeated measures. Association of these loci with capillary glucose and repeated measures of multiple complications of diabetes were also assessed.

Results: A major locus for HbA1c was found in the conventional treatment group near SORCS1 ($10q25.1$, $p = 7 \times 10^{-10}$), and it was also associated with mean glucose ($p = 2 \times 10^{-5}$). This was confirmed using A1C in the intensive treatment group ($p = 0.01$). Other loci achieved evidence close to genome-wide significance: $14q32.13$ (GSC) and $9p22$ (BNC2) in the combined treatment groups and $15q21.3$ (WDR72) in the intensive group. The association of these loci with complication risk was also assessed and SORCS1 was linked with hypoglycemia, whereas BNC2 with renal and retinal complications.

The association with glycemic control was replicated for SORCS1 in Genetics of Diabetes in Kidneys (GoKinD) study control subjects ($p = 0.01$) and for BNC2 in non-diabetic individuals.

Conclusion: A major locus for A1C and glucose in individuals with T1D is near SORCS1.

Hyperglycemia is a major player in the development of vascular complications of diabetes, through the activation of several metabolic pathways [11]. Landmark studies such as the DCCT and the EDIC have highlighted the key role of glycemic control in the preventing and/or slowing the development and progression of micro- and macrovascular complications.

It is intriguing to observe that reaching a good glycemic control requires variable efforts in different patients and that glycemic control is often quite consistent in a given individual with diabetes. These findings underline the potential role of a genetic background in modulating plasma glucose concentrations. The role of genetic factors is also supported by twin and family studies, which have shown the heritability of measures of glycemic control, such as HbA1c, in non-diabetic individuals [12].

The present study deals with the important issue of looking for genetic determinants of glycemic control in people with T1D, using longitudinal data collected during the DCCT. A major locus (rs1358030) for HbA1c and mean glucose levels was found near SORCS1, a gene with some previous evidence of an association with glycemic traits. This locus was also replicated in a separate population, the GoKinD control subjects, and it also showed evidence for association with hypoglycemia. Other loci achieving evidence close to genome-wide significance were GSC, BNC2, and WDR72. Among them, BNC2 was replicated in a non-diabetic population (MAGIC) and was also associated with renal and retinal complications.

Characterizing genes regulating glycemic control is fascinating and represents a major step forward in the management of patients with diabetes. In fact, the identification of individuals genetically predisposed to a poor metabolic control and therefore at higher risk for complications represents an important step in the long and fascinating way towards a personalized medicine, based on the concept that each patient should be considered as 'a single one', requiring a specific and personalized treatment plan, defined on the basis of his/her risk profile.

The clear definition of genetic determinants of glycemic control is not an easy task as it requires to clearly distinguish the effect of heritability from environmental influences including treatment itself.

New genes 2 Confirmation of T1D genes

In silico replication of the genome-wide association results of the Type 1 Diabetes Genetics Consortium

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Background: The Type 1 Diabetes Genetics Consortium (T1DGC) recently reported 22 novel T1D-associated loci identified by meta-analysis of three genome-wide association studies (GWASs) with a case-control design. However, the association of 10 of these 22 reported loci was not confirmed in the T1DGC family cohort. The aim of this study was to replicate the association in three independent GWAS cohorts to exclude potential bias from population stratification.

Methods: Three European-descent population samples were included: 483 cases and both parents, a case-control cohort of 514 cases and 2,027 controls, and an additional cohort of 1,078 cases and 341 controls from the dbGaP database. Among the 22 SNPs reported by the T1DGC, there were high-quality genotypes for 15; the remaining were imputed.

Results: T1D association was replicated in seven loci after Bonferroni correction for 22 independent hypotheses. An additional eight loci had nominal significance of $p < 0.1$ in the same direction. The genetic susceptibility conferred by non-HLA loci in the family cohort with 1 affected offspring was higher than the T1DGC multiplex families, whereas the frequency of HLA alleles in the multiplex families was higher.

Conclusion: This study replicated T1D association with at least as many of these novel loci as expected from the power of the sample size, thus supporting the validity of the new discoveries.

T1D is a complex disease resulting from the interaction of multiple genetic and environmental factors. Over the years many efforts have been made to understand its pathogenesis.

Several genetic determinants had been identified already before the era of genome-wide association studies (GWAS). The recent development of high-throughput single nucleotide polymorphism genotyping array technologies has enabled investigators to perform high-density GWAS in search of additional T1D loci and the results of many GWAS have been reported to data.

In last year chapter on T1D there were two important papers dealing with the genetics of T1D and the role of GWAS in allowing identification of a large number of loci associated with the disease [13]. The present study aimed to extend previous genetic findings in different populations.

The T1DGC reported 22 novel loci associated with T1D; however, 10 of these 22 loci were not replicated in the T1DGC family cohort, raising the point as to whether heterogeneity in the populations selected in the different studies could have influenced the results [14].

Therefore, in the present study the authors attempted to validate the 22 previous identified loci, using three European descendent population samples. T1D association with seven loci was validated with a $p < 4.55 \times 10^{-3}$, other eight loci had a $p < 0.01$, whereas seven loci were not replicated. Overall this study validated the T1D association previously reported by the T1DGC and highlighted the complexity of genetics of T1D and the need of further investigations to understand the true role of the identified loci in the pathogenesis of T1D.

New concerns

The lower the plasma glucose, the bigger the hippocampus

Hippocampal volumes in youths with type 1 diabetes

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Background: Hippocampal neurons have been suggested to be particularly vulnerable to glycemic excursions. The aim of this study was to assess the effect of hypoglycemia and hyperglycemia on hippocampal volume during brain development.

Methods: The study population was represented by 95 youths with T1D and 49 sibling control subjects aged 7–17 years, who underwent magnetic brain resonance imaging. Stereologic measurements of hippocampal volumes were performed in atlas-registered space to correct for whole brain volume. T1D youths were categorized as having 0 ($n = 37$), 1–2 ($n = 41$), or 3 or more ($3+; n = 17$) prior severe hypoglycemic episodes. Hyperglycemia exposure was estimated from median lifetime A1C, weighted for duration of diabetes.

Results: Greater exposure to severe hypoglycemia during childhood was associated with enlargement of the hippocampal volume ($F [3,138] = 3.6, p = 0.016$; $3+$ larger than all other groups, $p < 0.05$). In contrast, hyperglycemia exposure was not associated with hippocampal volumes, and the $3+$ severe hypoglycemia group still had larger hippocampal volumes after adjusting for age of onset and hyperglycemia exposure (main effect of hypoglycemia category, $F [2,88] = 6.4, p = 0.002$; $3+$ larger than all other groups, $p < 0.01$).

Conclusion: The increased hippocampal volume associated with severe hypoglycemia may be due to gliosis, reactive neurogenesis, or disruption of normal developmental pruning in the developing brain.

Diabetes can alter the function and structure of many organs, including the brain. Hyperglycemia and hypoglycemia can both alter the brain, damaging distinct areas. Data from animal studies have shown that hypoglycemia can selectively damage neurons in the medial temporal region, including

the hippocampus. However, there are limited data in humans and in particular on the effect of hypoglycemia on the developing brain of young people with diabetes.

Hershey et al. present interesting data, collected in a large group of 95 youths with T1D and 49 healthy children, on increased hippocampus volume in relation to severe hypoglycemic episodes. Hippocampal volume, measured with validated and unbiased stereologic methods, was strongly related to previous history of severe hypoglycemia, independently of potential confounders such as age, duration of diabetes, sex, and hyperglycemia. Interestingly, hyperglycemia, which is known to influence brain function, did not have any association with hippocampal volume. These data are unique as previous studies in adults with diabetes had shown no evidence of variation in hippocampal volume or, signs of neuronal death in patients with hypoglycemia.

The present findings of an increased hippocampal volume might indicate a pathological response to severe hypoglycemia, expressed as gliosis, reactive neurogenesis, disruption of normal developmental pruning or a compensatory response to damage. Further studies will likely clarify the underlining mechanisms. For the moment, the available data deserve reflection on how vulnerable the developing brain to hypoglycemia can be and how the detected changes can evolve over time and influence long-term neurocognitive function.

New mechanisms 1

Epigenetic modulation of the insulin gene

Insulin gene expression is regulated by DNA methylation

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Background: Insulin is a critical component of metabolic control, and as such, insulin gene expression has been the focus of extensive study. In this study the role of DNA methylation in the regulation of mouse and human insulin gene expression was investigated.

Methods: Genomic DNA samples from several tissues were bisulfite-treated and sequenced.

Results: Cytosine-guanosine dinucleotide (CpG) sites in both the mouse *Ins2* and human *INS* promoters are uniquely demethylated in pancreatic β -cells. Methylation of these CpG sites suppressed insulin promoter-driven reporter gene activity by almost 90% and specific methylation of the CpG site in the cAMP-responsive element (CRE) in the promoter alone suppressed insulin promoter activity by 50%. Methylation did not directly inhibit factor binding to the CRE in vitro, but inhibited ATF2 and CREB binding in vivo and conversely increased the binding of methyl-CpG binding protein 2 (MeCP2). In mouse embryonic stem cell cultures, the *Ins2* gene is fully methylated and becomes demethylated during differentiation into insulin-expressing cells in vitro.

Conclusion: Insulin promoter CpG demethylation may play a crucial role in β -cell maturation and tissue-specific insulin gene expression.

Epigenetics is a central process implicated in the control of gene expression, providing the mechanism by which the environment interacts with identical genotypes to produce different phenotypes. Recently, interest has been focused on the implication of this process in the pathogenesis of T1D, through regulation of key molecules and processes involved in β -cell development, survival, regeneration, function and autoimmune reactions [15].

In this paper, the emerging concept of 'epigenetic modifications' is assessed in relation to a key gene in the context of diabetes: the insulin gene. The authors found that methylation of this gene, as in general for other genes, is a mechanism of silencing its activity, whereas demethylation is associated with progressive expression of the gene and therefore increased insulin levels.

DNA methylation patterns of the insulin gene were assessed in human and mouse β -cells as well as in non- β -cells and in embryonic stem cells from the undifferentiated stage throughout the stages leading to insulin-expressing cells.

The human insulin gene appears to have nine CpG sequences in its promoter. Demethylation of these sites is important for both basal and stimulated insulin gene expression. In contrast, in non- β -cells these sites are completely or in part methylated. Methylation of these sites induces recruitment of CpG binding protein 2 and probably of other proteins, which can interfere with binding of other factors, with the consequent suppression of gene expression.

Another key finding of the study was that demethylation of the insulin promoter does not appear to be specifically involved in daily metabolic regulation, but it is more likely to be implicated in the development of insulin-producing β -cells. Demethylation of the insulin gene is a late step in the differentiation of the β -cell phenotype from embryonic stem cells.

These findings highlight the complexity of gene regulation in humans and help to explain the difficulties met by the scientists who are trying to produce β -cells in vitro, which should be then transplanted in patients with T1D. Up to now, a major problem encountered in this context has been how to obtain normal levels of insulin gene expression. The specific methylation/demethylation pattern that emerged from the present study is clearly a major aspect to consider and overcome in future studies, together with other potential epigenetic modulations of the insulin gene.

New mechanisms 2 Seeking β -cell biomarkers

A genomic-based approach identifies FXYD domain containing ion transport regulator 2 (FXYD2) γ as a pancreatic β -cell-specific biomarker

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Background: Non-invasive imaging of the pancreatic β -cell mass requires the identification of novel and specific β -cell biomarkers. In the present study a systems biology approach was used in order to identify potential β -cell markers.

Methods: A functional genomics strategy based on massive parallel signal sequencing and microarray data obtained in human islets, purified primary rat β -cells, non- β -cells and INS-1E cells was applied. Candidate biomarkers were validated and screened using established human and macaque (*Macacus cynomolgus*) tissue microarrays.

Results: After a series of filtering steps, 12 β -cell-specific membrane proteins were identified. For four of the proteins antibodies targeting specifically the human proteins and their splice variants were produced and allowed to confirm all four candidates as islet-specific in human pancreas. Two splice variants of FXYD domain containing ion transport regulator 2 (FXYD2), a regulating subunit of the Na⁺,K⁺-ATPase, were identified as preferentially present in human pancreatic islets. The presence of FXYD2 γ was restricted to pancreatic islets and selectively detected in pancreatic β -cells. Analysis of human fetal pancreas samples showed the presence of FXYD2 γ at an early stage (15 weeks). Histological examination of pancreatic sections from individuals with T1D or sections from pancreases of streptozotocin-treated *M. cynomolgus* monkeys indicated a close correlation between loss of FXYD2 γ and loss of insulin-positive cells.

Conclusion: This study suggests human FXYD2 γ as a novel β -cell-specific biomarker.

In the present study the application of a systems biology approach led to the identification of a specific new β -cell biomarker, which could have important implications for assessing β -cell mass with non-invasive techniques. The possibility of assessing β -cell mass in humans represents an essential tool in studies aiming at better understanding the pathogenesis of the diseases as well as in evaluating the effect of emerging preventive strategies, directed at reducing β -cell loss before disease onset.

This study used a step-by-step approach consisting on functional genomics based on parallel sequencing and microarray data obtained from human islets, purified primary rat islet β -cells and non- β -cells. Starting from 950 islet-specific genes, 114 were then identified as expressed at the membrane level and 44 of them were found to be preferentially expressed in β -cells. Out of these 44 genes, 12 were further selected based on their non-responsiveness to inflammatory cytokines. The best candidate biomarker for β -cells was FXVD2, with two of its three variants expressed in the human β -cell and one, FXVD2 γ a, exclusively expressed in these cells. This molecule represents a regulatory subunit of the ubiquitously distributed Na⁺,K⁺-ATPase [16]. Expression of this FXVD2 γ a isoform emerged to be an early embryonic event and its levels decreased in parallel with β -cell mass loss. These results suggest this biomarker as a potential useful one for non-invasive imaging and quantification of pancreatic β -cell mass. Further studies will hopefully confirm these findings and the usefulness of this biomarker firstly in the research setting and then also in clinical practice.

New mechanisms 3 Time to focus on antigen presentation!

Targeted regulation of self peptide presentation prevents type 1 diabetes in mice without disrupting general immunocompetence

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Background: Peptide loading of MHC class II (MHCII) molecules is directly catalyzed by the MHCII-like molecule HLA-DM (DM). Another MHCII-like molecule, HLA-DO (DO), associates with DM, thereby modulating DM function and dampening presentation of self antigens.

Methods: In order to test the idea that DO modulation of the MHCII self peptide repertoire mediates self-tolerance, NOD mice (a mouse model for T1D) that constitutively overexpressed DO in DCs (referred to herein as NOD.DO mice) were generated.

Results: Diabetes development was completely blocked in NOD.DO mice. NOD.DO animals selected a diabetogenic T-cell repertoire, the numbers and function of Tregs were normal, and therefore their immune system function was equivalent to that in NOD mice. However, NOD.DO DCs presented an altered MHCII-bound self peptide repertoire, thereby preventing the activation of diabetogenic T cells and subsequent diabetes development.

Conclusion: DO expression can shape the overall MHCII self peptide repertoire to promote T-cell tolerance.

Up to now much of the focus in the pathogenesis of T1D has been directed towards T lymphocytes, being T1D a T-cell-mediated autoimmune disease. However, an important aspect in the development of self-reactive lymphocytes is represented by reaction with self antigens presented by specific antigen presenting cells, such as dendritic cells, in the context of the MHCII molecules [17].

The present study clearly shows that subtle changes in MHCII antigen presentation can prevent disease development, therefore proposing the manipulation of antigen presentation by dendritic cells as a new potential target toward which preventive strategies should be directed.

Peptide loading of MCHII is catalyzed in late endosomal and lysosomal compartments of cells by the catalytic action of human HLA-DM. In B cells, dendritic cells and thymic epithelial cells, the peptide loading of class II molecules is modified by the expression of the non-classical class II molecule, HLA-DO [17]. The biological role of HLA-DO-mediated regulation of DM activity in vivo remains unknown; however, it has been postulated that DO expression dampens presentation of self antigens, thereby preventing inappropriate T-cell activation that ultimately leads to autoimmunity [18]. In the present study performed in a NOD.DO mice model, the development of diabetes was prevented by HLA-DO expression in dendritic cells. This protection appeared to be due to an inefficient presentation of self antigens by dendritic cells overexpressing HLA-DO, therefore maintaining periph-

eral tolerance and confirming the above discussed hypothesis. These data suggest that when investigating the pathogenesis of T1D it is important not only to direct efforts towards autoreactive T lymphocytes, but consider the numerous players involved in the autoimmune reactions.

Clinical trial, concepts revised To treat or not to treat?

Renal and retinal effects of enalapril and losartan in type 1 diabetes

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Background: It is unclear whether progression of nephropathy and retinopathy in people with T1D is slowed by early administration of drugs blocking the renin-angiotensin system (RAS).

Methods: A multicenter, controlled trial involving 285 normotensive patients with T1D and normoalbuminuria, randomly assigned to receive losartan (100 mg daily), enalapril (20 mg daily), or placebo and followed for 5 years, was performed. The primary endpoint was a change in the fraction of glomerular volume occupied by mesangium in kidney-biopsy specimens. The retinopathy end point was a progression of two steps or more.

Results: A total of 90 and 82% of patients had complete renal biopsy and retinopathy data, respectively. Change in mesangial fractional volume per glomerulus over the 5-year period did not differ between the placebo (0.016 units) and the enalapril (0.005, $p = 0.38$) or the losartan group (0.026, $p = 0.26$), nor were there significant differences in the other renal structural variables. The 5-year cumulative incidence of microalbuminuria was 6% in the placebo group; the incidence was higher with losartan (17%, $p = 0.01$) but not with enalapril (4%, $p = 0.96$). Compared with placebo, enalapril reduced the odds of retinopathy progression by 65% (odds ratio [95% confidence interval]: 0.35 [0.14–0.85]) and losartan by 70% (0.30 [0.12–0.73]), independently of blood pressure changes. There were three biopsy-related serious adverse events that completely resolved. Chronic cough occurred in 12 patients receiving enalapril, 6 receiving losartan, and 4 receiving placebo.

Conclusion: Early blockade of the RAS in patients with T1D did not slow nephropathy progression but slowed the progression of retinopathy.

Several studies, mainly performed in adults, have shown that treatment with angiotensin-converting enzyme inhibitors (ACEIs) reduces the rate of progression and can even promote regression of microalbuminuria [19], independently from the effect on blood pressure. ACEIs can also have a significant effect on other diabetic microvascular complications, such as retinopathy [20] and potentially cardiovascular disease [21].

The study by Mauer and colleagues does not confirm the previously observed beneficial results of ACEIs therapy in patients with diabetic nephropathy and highlights the concept that blockers of the RAS do not necessarily change renal pathology in initially normotensive normoalbuminuric subjects. The Renin-Angiotensin System Study (RASS) is up to now the largest long-term study assessing the effect of a 5-year blockage of the RAS system in people with T1D. In this study, two different strategies of inhibiting the RAS were evaluated: ACEIs and angiotensin receptor inhibitors (ARBs). In addition, a novelty of the present study was the primary study endpoint, which was represented by early renal structural alterations, assessed through renal biopsies performed at baseline and 5 years later. In contrast, the majority of previous studies had as main study endpoint changes in albumin excretion.

Surprisingly, the study not only demonstrated that treatment with ACEIs or ARBs did not influence any structural renal parameter but it also showed that losartan increased the incidence of microalbuminuria when compared to placebo (17 vs. 6%), whereas enalapril did not have any effect on this secondary study endpoint.

In contrast, inhibition of the RAS was associated with a 65–70% reduction in retinopathy progression, independently of changes in blood pressure, in patients with no sign or minimal pre-proliferative retinopathy at baseline.

How can this different effect on the two vascular complications be explained? As pointed out also in the accompanying editorial of this paper, the pathogenesis of complications of diabetes is heterogeneous, with an interplay of several mechanisms, which can differ or have a different effect in the different microcirculations of the kidney and retina, particularly during the early stages of diabetic complications.

With regard to differences from previous studies, the lack of an effect on renal outcomes might be related to the fact that intervention was started at an early stage of renal pathology, whereas in other investigations a beneficial effect was seen when applied at a more advanced stage of diabetic nephropathy. Again, this could implicate a different role of RAS in different stages of nephropathy.

Clinical trial, new treatments On the way towards the artificial pancreas

Manual closed-loop insulin delivery in children and adolescents with type 1 diabetes: a phase 2 randomized crossover trial

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Background: The effect of a closed-loop system, linking continuous glucose measurements to insulin delivery, on overnight blood glucose control was assessed in young people with T1D.

Methods: Three randomized crossover studies were performed in 19 patients (age: 5–18 years; mean (SD) T1D duration: 6.4 (4.0) years). In study 1 (n = 13), a standard continuous subcutaneous insulin infusion was compared with closed-loop insulin delivery; in study 2 (n = 7) closed-loop delivery was assessed after rapidly and slowly absorbed meals; in study 3 (n = 10) a comparison was performed between closed-loop delivery and standard treatment after exercise. During closed-loop nights, glucose was assessed every 15 min and values included into a control algorithm calculating rate of insulin infusion, and a nurse adjusted the insulin pump. During control nights, patients' standard pump settings were applied. Primary outcomes were time for which plasma glucose concentration was 3.91–8.00 or 3.90 mmol/l or lower.

Results: Primary outcomes did not differ significantly between treatment groups in the three studies considered individually: study 1 (target range, median 52% closed loop vs. 39% standard treatment, p = 0.06; ≤ 3.90 mmol/l, 1 vs. 2%, p = 0.13), study 2 (target range, rapidly 53% vs. slowly absorbed meal 55%, p = 0.97; ≤ 3.90 mmol/l, 0 vs. 0%, p = 0.16), and study 3 (target range 78% closed loop vs. 43% control, p = 0.0245, not significant at corrected level; ≤ 3.90 mmol/l, 10 vs. 6%, p = 0.27). A secondary analysis of pooled data (study 1 + study 3) documented increased time in the target range (60 vs. 40%; p = 0.0022) and reduced time for which glucose concentrations were 3.90 mmol/l or lower (2.1 vs. 4.1%; p = 0.0304). During closed-loop delivery there were no events with plasma glucose concentration < 3.0 mmol/l compared with nine events during standard treatment.

Conclusions: Overnight manual closed-loop insulin delivery can improve glucose control and reduce risk of nocturnal hypoglycemia in young patients with T1D.

Hovorka et al. report the results of three randomized crossover studies performed in children and adolescents with T1D, where the effect of a manual closed-loop insulin delivery system on overnight blood glucose was assessed in comparison to a standard continuous insulin infusion. In addition, the performance of this system was investigated in relation to a variable-content evening meal and moderate-intensity evening exercise.

Insulin therapy has been marked by unforgettable milestones starting from insulin discovery and followed, during most recent years, by the advent of insulin analogues and the implementation of pump therapy and continuous glucose monitoring. However, nowadays, treatment is still problematic and exogenous insulin administration is still far away from mimicking the endogenous insulin pattern. In addition, although intensive insulin therapy is the cornerstone of an optimal management of diabetes, aiming at reducing complication risk, the associated risk of hypoglycemia cannot be underestimated. Closed-loop insulin systems, through the combination of a continuous glucose monitor, a control algorithm and an insulin pump, aim at resembling an artificial pancreas [22].

Hovorka's study shows that this system can achieve a safe and good overnight glucose control. By combining the data from studies 1 and 3, the authors found that closed-loop insulin delivery was associated with an increased likelihood of plasma glucose concentrations to be in the target range and reduced frequency of low glucose values, particularly after midnight when the system appears to have reached full effectiveness.

Although further assessment and improvements of the system are required in the next years, the present results represent an important step forward in a still complex way towards the development and implementation of an artificial pancreas, which could not only ease daily life, but also solve the fears and consequences related to uncontrolled hyperglycemic and hypoglycemic episodes in children with T1D.

New hope 1 B-cell depletion as a novel therapy for T1D

Rituximab, B-lymphocyte depletion, and preservation of β -cell function

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Background: There is growing evidence that B lymphocytes play a role in many T-lymphocyte-mediated diseases, such as T1D. The aim of this phase 2 study was to evaluate the role of B-lymphocyte depletion with rituximab, an anti-CD20 monoclonal antibody, in patients with T1D.

Methods: In this randomized, double-blind study, 87 patients (aged 8–40 years) with newly diagnosed T1D were assigned to receive infusions of rituximab or placebo on days 1, 8, 15, and 22 of the study. The primary outcome, assessed 1 year after the first infusion, was the geometric mean area under the curve (AUC) for the serum C-peptide level during the first 2 h of a mixed-meal tolerance test. Secondary outcomes included safety and changes in HbA1c and insulin dose.

Results: At 1 year, the mean AUC for the level of C peptide was significantly higher, whereas HbA1c and insulin dose were lower in the rituximab than in the placebo group. A lower rate of decline in C-peptide levels between 3 and 12 months was detected in the rituximab than in the placebo group. CD19+ B lymphocytes were depleted in patients in the rituximab group, but levels increased to 69% of baseline values at 12 months. There was no increase in infections or neutropenia with rituximab, although patients in the rituximab group were more likely to experience grade 1 or 2 adverse events after the first infusion.

Conclusions: A four-dose course of rituximab partially preserved β -cell function over a period of 1 year in patients with T1D, suggesting a role of B lymphocytes in the pathogenesis of T1D.

T1D is an autoimmune disease in which T lymphocytes mediate damage to pancreatic β -cells. However, as for other T-cell-mediated diseases, a potential role for B lymphocytes has also been supposed and this represents the starting point for the hypothesis of the present study [23].

In this randomized double-blind study, depletion of B lymphocytes with the anti-CD20 monoclonal antibody rituximab was able to preserve β -cell function in patients with newly diagnosed T1D. HbA1c and insulin dose were reduced in the rituximab group, and these differences were explained by pres-

ervation of C-peptide levels. Although there was a higher rate of infusion reactions, there was no increased risk of neutropenia and infections, therefore supporting the safety, at least in the short term, of this treatment.

Rituximab is a drug approved for treatment of B-cell lymphomas and its efficacy has also been shown in several autoimmune diseases [24]. In a previous animal study, treatment with anti-CD20 antibodies was associated with B-cell depletion, and significantly delayed and/or reduced the onset of diabetes [25].

The present study provides new hopes for preserving β -cell function in people treated soon after diagnosis. This is of relevant importance as a residual insulin capacity has been associated with a better metabolic control and reduced complication risk.

How can B-cell depletion be protective against diabetes? In the context of diabetes, B lymphocytes act through the production of autoantibodies, the hallmark of autoimmunity. However, it is controversial whether autoantibodies exert a main pathogenetic role in T1D. It is more likely that they enhance or facilitate T1D development without having a main role in its induction. However, B cells can also act as islet antigen-presenting cells for autoreactive T cells and B-cell depletion is associated with the generation of regulatory T and B cells, which could modulate the immune process [24, 25]. Given the demonstrated importance of both T and B cells in diabetes pathogenesis, modulation of both arms of the immune response may represent a key strategy for the development of protective immunotherapies. Further research in this area is mandatory to confirm the present findings, clarify mechanisms beyond them and to assess the long-term safety related to immunosuppressive therapies.

New hope 2

Halting glucose excursions in youths with T1D

Ramlintide lowered glucose excursions and was well tolerated in adolescents with type 1 diabetes: results from a randomized, single-blind, placebo-controlled, crossover study

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J Pediatr 2009;155:369–373

Background: In adults with type 1 and type 2 diabetes, pramlintide reduce gastric emptying, glucose excursions, improve glycemic control and reduced weight gain. In this study the pharmacokinetics, pharmacodynamics, safety, and tolerability of pramlintide in adolescents with T1D were assessed.

Methods: 12 subjects (9 females, 3 males, age 12–17 years; A1C, 8.4%; body mass index, 25 kg/m²) were randomized to pramlintide (15 or 30 μ g) or placebo administered before a standardized breakfast. Insulin lispro (50% of usual mealtime dose) was injected separately. Acetaminophen (1,000 mg) was administered orally to provide an indicator of gastric emptying rate.

Results: Complete data were available for 9 participants, where plasma pramlintide concentrations increased in a dose-dependent manner. Mean peak plasma concentration (C_{max}) (15- μ g dose, 93 $\pm$ 9 pg/ml; 30- μ g dose, 202 $\pm$ 21 pg/ml) occurred approximately 0.3 h (median time to peak concentration) after administration. Pramlintide reduced incremental area under the concentration curve (AUC(0–3 h)) for glucagon and glucose versus placebo (glucagon: 15- μ g dose, 4 $\pm$ 7 pg $\cdot$ h/ml; 30- μ g dose, 5 $\pm$ 7 pg $\cdot$ h/ml; placebo, 35 $\pm$ 9 pg $\cdot$ h/ml; glucose: 15- μ g dose, 129 $\pm$ 43 mg $\cdot$ h/dl; 30- μ g dose, 132 $\pm$ 37 mg $\cdot$ h/dl; placebo, 217 $\pm$ 56 mg $\cdot$ h/dl). Acetaminophen C(max) decreased with pramlintide; median T(max) was delayed by approximately 2.6- to 3.8-fold. Pramlintide was well tolerated, and no treatment-related adverse events occurred.

Conclusions: In adolescents with T1D, pramlintide showed a similar pharmacokinetic profile as in adults and was able to reduce postprandial glucagon and glucose excursions and slow gastric emptying. Larger and longer term studies are warranted to confirm and extend the results of this study.

Achieving a good glycemic control is the cornerstone of treatment of diabetes but also a major challenge, particularly in adolescents with T1D. Adolescence is a period characterized by several physiological and psychological changes, which can influence glycemic control. The DCCT study clearly highlighted this issue by showing a 1% difference in HbA1c and a higher risk of hypoglycemia and weight gain between the adolescent and adult cohorts.

Adjunct therapies to improve glycemic control are therefore particularly warranted in this age group. But, which therapies should be considered? In healthy subjects, glucose homeostasis is the results of an interplay of several hormones, including insulin, glucagon, glucagon-like peptide-1 and amylin [26]. Therefore, in people with diabetes, not only there is a lack of endogenous insulin production, but the secretion and/or the action of these other hormones may be impaired and contribute to the overall metabolic imbalance.

In adults with diabetes, pramlintide, a synthetic analog of the pancreatic hormone amylin, has been shown to decrease HbA1c and postprandial glucose excursions as well as to reduce weight gain and gastric emptying [27]. Preliminary data in adolescents with T1D have shown that this molecule can delay gastric emptying, inhibit glucagon secretion and improve postprandial glucose excursions [28].

Chase et al. present a single-blind randomized controlled cross-over trial aiming at establishing the pharmacokinetics and safety profile of two doses of pramlintide, 15 and 30 µg, in a small group of 12 adolescents. Plasma concentrations of pramlintide were dose-dependent, whereas there was no such an effect on time for reaching peak concentration and duration of effect. Both doses of pramlintide were equally able to decrease glucagon release, reduce plasma glucose excursions and delay gastric emptying. No significant adverse effects were detected.

Overall this study shows that in adolescents pramlintide has similar pharmacokinetics and safety profile than in adults and could represent a valid adjunct therapy, particularly in adolescents with fluctuating blood glucose control. Further larger and longer term studies are required and hopefully they will confirm and strengthen the findings of the present study.

Concepts revised

Childhood- vs. adulthood-onset diabetes: does it matter?

Age at onset and the risk of proliferative retinopathy in type 1 diabetes

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Background: The aim of this study was to assess how age at the onset of T1D influences the long-term risk of developing proliferative retinopathy.

Methods: Fundus photographs and/or ophthalmic record were available for 1117 consecutively recruited patients taking part to the FinnDiane Study. The risk of proliferative retinopathy was studied in age at onset groups 0–4, 5–14 and 15–40 years.

Results: The mean durations to proliferative retinopathy were 24.3 (22.7–25.9) years in 0–4 group, 20.1 (19.2–21.1) years in 5–14 group, and 21.6 (19.8–23.3) years in 15–40 group ($p < 0.001$). In a Cox regression model, after adjusting for potential confounders, the highest risk of proliferative retinopathy was observed in 5–14 group (HR 1.90 [95% CI 1.45–2.48], $p < 0.001$). The long-term risk of proliferative retinopathy did not differ between those diagnosed aged 0–4 years compared with 5–14 years ($p = 0.2$). Risk of proliferative retinopathy was significantly higher in the age at onset group <15 years than in the age at onset group ≥ 15 years (HR 1.82 [95% CI 1.40–2.36], $p < 0.001$).

Conclusions: An early age at onset of T1D confers a longer time free of proliferative retinopathy. However, this advantage disappears over time. The highest risk for developing proliferative retinopathy is in age at onset group 5–14, whereas the lowest risk is in the age at onset group 15–40 years.

An early onset of diabetes has been for a long time thought to be protective with respect to the development of long-term vascular complications [29]. In this recent study, Hietala et al. revise this

concept and report that, although patients diagnosed at a young age (0–4 years) have a longer time free of proliferative retinopathy, this advantage gradually disappears over time and, after about 30-year diabetes duration, their risk of developing proliferative retinopathy is comparable to that of patients diagnosed when aged 5–14 years. These results are similar to those reported by Amin et al. [30] in 2008, for risk of microalbuminuria in a longitudinal cohort of people with childhood-onset diabetes. In Amin's study, children with an early onset of diabetes showed a silent period, but after 15 years of diabetes duration the risk of developing microalbuminuria was similar between subjects diagnosed with diabetes before 5 years of age and those diagnosed between 5 and 11 years of age or after puberty.

Taken together, the results of these two studies suggest that an early age at the onset of diabetes does not protect from complications, but it does affect the age at which complications are first detected.

Hietala and colleagues offer several suggestions on the mechanisms which could explain the delayed onset of complications in their youngest age group. One hypothesis is that behavioral factors might play a role, so that younger children learn easier good self-care skills, which then lead to better metabolic control. A more stringent diabetes management might be another implicated factor. In addition, the negative effect of puberty on glycemic control needs to be considered as a potential accelerator of complications onset.

Another important point raised from the Hietala's study was the lowest risk detected in patients diagnosed at an age between 15 and 40 years. This is likely related to a less aggressive form of diabetes characterizing this age group. In particular this could be explained by a better preservation of β -cell function and of C-peptide levels, factors which have been associated with a decreased incidence of complications [31].

Age at onset of type 1 diabetes in parents and recurrence risk in offspring

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Background: The aim of this study was to assess the recurrence risk of T1D in the offspring of parents with adult-onset (15–39 years) T1D and the transmission of diabetes within a continuum of parental age at onset of diabetes from childhood to adulthood.

Methods: Diabetes status of all offspring ($n = 9,636$) in two Finnish cohorts of parents with T1D was defined until the end of year 2007. Cumulative incidences of T1D among the offspring were estimated, and several factors contributing to the risk were assessed.

Results: During 137,455 person-years, a total of 413 offspring were diagnosed with type 1 diabetes. The cumulative incidence by 20 years was 4.0% (95% CI 3.1–4.8) for the offspring of parents with adult-onset diabetes, with a similar risk in offspring of diabetic mothers and fathers. The cumulative incidence decreased in parallel with the increase in age at onset of diabetes in the fathers, whereas the risk did not change with age at onset in the offspring of mothers with T1D. However, the reduced risk in the maternal offspring was most pronounced in the daughters of the mothers with a diagnosis age <10 years.

Conclusion: T1D transmission ratio distortion is strongly related to the sex and age at onset of diabetes in the diabetic parents.

Up to now, studies have been assessing the risk of diabetes recurrence in offspring of parents with onset of diabetes during childhood. The majority of these studies have shown a higher risk when the father is affected by T1D than when the mother is the index case.

The present study represents a step forwards in the understanding of transmission of diabetes as it assesses this issue within a continuum of parental age at onset from childhood to adulthood. The study is based on population-based cohorts from Finland: 3,881 offspring in the late-onset cohort, diagnosed between 15 and 39 years of age and 5,821 in the early-onset cohort, diagnosed before the age of 15 years. T1D developed in 318 offspring in the early-onset cohort and 97 in late-onset cohort. In the late-onset cohort, where the cumulative risk was 4% by 20 years, the risk in the offspring did not differ by sex of the parents: 4.2% in the offspring of mothers vs. 3.8% in the offspring of fathers.

Interestingly, whereas in the offspring of fathers from the late-onset cohort the risk decreased with age at diagnosis (HR = 2.9 for 15–19 years vs. 35–39), this was not the case for the offspring of mothers. This age-related trend was even more evident when both the early- and late-onset cohorts were considered together, resulting in a declining risk in the offspring of fathers with increased age at onset. For the mothers, no such trend was detected, although when considering only the offspring of mother diagnosed before 10 years of age girls showed a higher risk than boys.

The findings of the present study confirm the concept that differences in the genetic, autoimmune and clinical aspects exist between childhood versus adulthood-onset diabetes. In particular, the results of this study are in line with the concept that age at onset is an indicator of genetic susceptibility. Therefore, an earlier onset underlines a stronger genetic component, and this is clearly evident from the pattern of risk in the offspring of diabetic fathers. Although in previous studies the lower risk in offspring of mothers was explained by a certain protection of the diabetic intrauterine environment, this protection cannot be claimed for offspring of mothers with adult-onset diabetes.

Food for thought Can we improve islet transplantation success?

High-mobility group box 1 is involved in the initial events of early loss of transplanted islets in mice

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Background: Islet transplantation for the treatment of T1D is limited in its clinical application mainly due to early loss of the transplanted islets, resulting in low transplantation efficiency. NKT cell-dependent IFN- γ production by Gr-1+CD11b+ cells is essential for this loss, but the upstream events in the process remain undetermined.

Methods: A mouse model of diabetes was used to assess the involvement of high-mobility group box 1 (HMGB1) in the initial events of early loss of transplanted islets.

Results: A major source of HMGB1 is represented by pancreatic islets, from where this protein is released into the circulation soon after islet transplantation. Treatment with an HMGB1-specific antibody prevented the early islet graft loss and inhibited IFN- γ production by NKT cells and Gr-1+CD11b+ cells. Moreover, mice lacking either of the known HMGB1 receptors TLR2 or receptor for advanced glycation end products (RAGE), but not the known HMGB1 receptor TLR4, failed to exhibit early islet graft loss. HMGB1 stimulated hepatic mononuclear cells (MNCs), through an upregulation of CD40 expression and an increased production of IL-12 by DCs, leading to NKT cell activation and subsequent NKT cell-dependent augmented IFN- γ production by Gr-1+CD11b+ cells. Treatment with either IL-12- or CD40L-specific antibody prevented the early islet graft loss.

Conclusion: HMGB1 and related activated pathways play an important role in early islet loss and represents a potential target for intervention to improve the efficiency of islet transplantation.

Islet transplantation is a promising procedure for the cure of diabetes. However, low efficiency of islet transplantation has been a major obstacle limiting its clinical applications. Early loss of transplanted islet is a key problem to be solved [32]. Animal models have shown that NKT cell-dependent IFN- γ production by Gr-1+CD11b+ cells is an important mediator of this early loss [33].

The present study represents a step forward in understanding this process, focusing on the role of HMGB1, a protein which appears to play a key role in response to tissue damage and is released by inflammatory cells, such as dendritic cells, NK cells, and macrophages [34]. Through a series of elegant and detailed experiments in a mouse model of diabetes, the authors reached some key conclusions. They found that a major source of HMGB1 is represented by pancreatic islets, where the protein is localized mainly in nuclei and its plasma levels increase soon after transplantation, therefore repre-

senting a potential early marker of transplant failure. HMGB1 activates the production of inflammatory cytokines including IL-12 and INF- γ . In particular, based on receptor expression pattern, the study showed that the first cell line target for HMGB is represented by dendritic cells, where they stimulate IL-12 production, which in turn could stimulate NKT-dependent INF- γ production. These inflammatory cytokines in turn further contribute to damage of the transplanted islets. The identification and definition of this complex network represents an important discovery, which highlights potential new targets for interventions aiming at increasing the rate of success of islet transplantation.

Reviews

Mitochondria-mediated cell death in diabetes

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Overview: Mitochondrial dysfunction play a role in the pathogenesis of a wide range of diseases that involve disordered cellular fuel metabolism and survival/death pathways, including neurodegenerative diseases, cancer and diabetes. Cytokine, virus recognition and cellular stress pathways converging on mitochondria cause apoptotic and/or necrotic cell death of β -cells in T1D. In addition, mitochondrial dysfunction underlies both the functional derangement of glucose-stimulated insulin secretion and stress-induced apoptotic/necrotic β -cell death, which characterize type 2 diabetes. In this review a summary of the main findings supporting such a key role of the mitochondria in β -cell death are summarized.

Mitochondria, the cellular aerobic bioenergy production sites, are involved in a variety of metabolic activities, and there are several lines of evidence showing a key roles of these intracellular organelles in the pathogenesis not only of diabetes but also of its complications [35]. Interestingly, mitochondrial increased oxidant generation seems to play an important role also in the context of the 'metabolic memory'. In fact, increased mitochondrial superoxide production consequent to hyperglycemia can induce not only immediate effects, but it might also damage mitochondrial DNA and proteins, leading to synthesis of altered mitochondrial respiratory channel subunits, which could produce increased amount of superoxide, even in presence of physiological glucose levels [35].

In this review the authors report and discuss the current level of evidence supporting a key role of mitochondria in β -cell death in the context of both T1D and type 2 diabetes. Although the pathogenesis of the two diseases is distinct, β -cell death in both conditions appears to be mediated by a common final mechanism, represented by mitochondrial membrane permeation. In T1D, β -cell death is directly linked to the autoimmune process, whereas in type 2 diabetes metabolic stress induces β -cell apoptosis/necrosis.

In the context of T1D, the main soluble mediator of β -cell apoptosis produced by CD4+ T cells and macrophages are IL-1 β , INF- γ and TNF- α , which stimulate NF- κ B activation, that in turn mediates the pro-apoptotic signal. Also, CD8+ T-cell-mediated β -cell death appears to be linked to mitochondria, through the involvement of bcl-2 family. Additional mechanisms linking β -cell death in T1D with mitochondria dysfunction are activation of poly(ADP-ribose) polymerase and NO competition for molecular oxygen at the level of complex IV of the mitochondria. Based on these data, mitochondria appear to be an important cellular component towards which further research should be directed to clarify aspects of the pathogenesis of diabetes.

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