Type 1 Diabetes: Clinical and Experimental

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The continuous rise in the incidence of type 1 diabetes (T1D), particularly in younger children, has been a constant theme highlighted in all previous *Yearbook* chapters on T1D. It is impossible not to reiterate this concept this year, given that it represents a major epidemiological issue and the driving force for the studies reported in this chapter.

Several interesting basic and clinical studies have been published in 2011–2012 and some of the most interesting ones, dealing with a wide range of aspects associated with T1D, from pathogenesis to treatment, are presented. Progress has been made in terms of the understanding of the pathogenesis of T1D and how changes in environmental factors can interact with the individual genetic background in explaining the rising number of new cases of T1D. Changes in the immunophenotype of patients with T1D seem to have occurred over time, moving towards a more mature and aggressive immune response. Peripheral blood monocyte expression profiles have emerged to be a clinical useful marker to stratify patients with recent-onset T1D. New β -cell-specific markers, which could be invaluable for diagnostic and intervention purposes, have also been identified. Clinical trials have been assessing new potential treatment strategies, such as immune treatments as well as new insulin analogues. Genetic studies have become more and more complex, by including interaction models which allow a better understanding of the role of genetic changes at single or multiple loci. In addition, the potential role of epigenetic changes has also been explored. Exciting has been the discovery of genetic manipulations which can influence insulin secretion, or even turn some gut cells into insulin-producing cells.

From a clinical point of view, interesting findings have been the higher risk of T1D associated with cesarean section in combination with genetic susceptibility, the beneficial effect of intensive insulin treatment on glomerular filtration rate and the feasibility of behavioral interventions in the management of adolescents with T1D. Last but not least, the 'new hope' section is, as last year, dedicated to the progress in the field of islet transplantation.

Many articles presented in this chapter will be followed by new studies aiming at confirming specific findings or extending them and leading to more discoveries, which will hopefully help prevent T1D or at least improve management, reducing complication risks and improving the prognosis of people affected by this disease.

Mechanism of the year Turning gut cells into β -cells

Generation of functional insulin-producing cells in the gut by Foxo1 ablation

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Nat Genet 2012;44:406–412

Background: Restoration of regulated insulin secretion is the ultimate goal of therapy for T1D.

Methods and Results: Somatic ablation of Foxo1 in Neurog3+ enteroendocrine progenitor cells gave rise to gut insulin-positive (Ins+) cells that expressed markers of mature β -cells and secreted bioactive insulin as well as C-peptide in response to glucose and sulfonylureas. Lineage tracing experiments showed that gut Ins+ cells arose from Foxo1-deficient cells. Inducible Foxo1 ablation in adult mice also resulted in the generation of gut Ins+ cells. Interestingly, after β -cell ablation by the toxin streptozotocin, gut Ins+ cells were able to regenerate and produce insulin, thus reversing hyperglycemia in mice.

Conclusions: The data indicate that Neurog3+ enteroendocrine progenitors require active Foxo1 to prevent differentiation into Ins+ cells. Foxo1 ablation in gut cells might become a key approach to restore insulin production in T1D.

Regeneration of β -cells and consequently insulin secretion is the fundamental goal for fighting T1D. Talchai et al. reports the very interesting finding on how loss of the transcriptional factor Foxo1 in Neurog3+ enteroendocrine progenitor cells can convert them into cells able to synthesize, process and secrete insulin as well as C-peptide. Following Foxo1 inactivation, enteric cells express markers typical of β -cells, such as Pdx1, MafA and Nkx6.1, and respond to β -cell secretagogues, such as glucose and sulfonylureas. Even more exciting was the finding that in mice gut, Ins+ cells derived from Neurog3+ progenitors can regenerate after streptozotocin treatment, differently from other cell lines previously shown to be able to convert into insulin-producing cells, but inexorably destroyed by streptozotocin. Therefore, not only inactivation of Foxo1 converts the fate of a subset of gut cells into insulin-producing cells, but can even confer them the potential of regenerating after a toxic hit.

Overall these data suggest that Foxo1 is a key factor required for suppressing a pancreatic β -cell-like program in enteroendocrine cells and, modulation of this factor appears to be a promising way for regenerating β -cells in the context of T1D.

These findings are promising and highlight new potential resources to be used when attempting to regenerate β -cells.

New paradigms 1

A monocyte signature as a biomarker of T1D progression

Peripheral blood monocyte gene expression profile clinically stratifies patients with recent-onset type 1 diabetes

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Diabetes 2012;61:1281–1290

Background: There is a great need to identify novel biomarkers of disease progression after T1D onset.

Methods: Peripheral blood (PB) monocyte gene expression was profiled in 6 healthy subjects and 16 children with T1D within 3 months from diagnosis.

Results: Monocyte expression profiles clustered into two distinct subgroups, representing mild and severe deviation from healthy control subjects. Patients with strongly divergent monocyte gene expression had significantly higher insulin dose-adjusted HbA_{1c} levels during the first year, compared with patients with mild deviation. The diabetes-associated expression signature identified multiple perturbations in pathways controlling cellular metabolism and survival, including endoplasmic reticulum and oxidative stress. Quantitative PCR (qPCR) of a 9-gene panel correlated with glycemic control in 12 additional recent-onset patients. The qPCR signature was also detected in PB from healthy first-degree relatives.

Conclusion: A PB gene expression signature correlates with glycemic control in the first year after T1D diagnosis and is present in at-risk subjects. These data suggest that monocyte phenotype might be a candidate biomarker for disease progression pre- and post-onset. In addition, systemic stresses appear to be contributors to innate immune function in T1D.

Searching for biomarkers of progression after T1D onset is of utmost importance to identify patients with different levels of severity of the disease, which could determine faster or slower progression rates and different risk of complications. Such biomarkers could also be particularly useful for clinical trials aiming at preventing T1D progression.

In this context, the present study was able to demonstrate that monocyte gene expression can help in distinguishing newly diagnosed patients with a different progression rate. The same expression profile could predict progression in subjects at risk for T1D before the onset of the disease. A severe deviation from a healthy monocyte expression profile, which was found in 50% of newly diagnosed children, was associated with a worse glycemic control and higher insulin requirements during a follow-up period up to 1 year after diagnosis. This might also determine a higher risk of complications in the long-term. Interestingly, this monocyte profile was characterized by upregulation of genes implicated in cellular stress, mainly oxidative and endoplasmic reticulum stress, and downregulation

of genes involved in cellular energy and metabolite production, thus highlighting an underlying high susceptibility or exposure to physiological stress.

Overall, these data suggest that monocyte phenotype might be a candidate biomarker for disease progression pre- and post-onset in T1D, which could allow a better patient stratification on the basis of different levels of progression risk and therefore potentially guide intervention strategies on a personalized basis.

New paradigms 2

Emerging new β -cell markers

Novel pancreatic β -cell-specific proteins: antibody-based proteomics for identification of new biomarker candidates

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J Proteomics 2012;75:2611–2620

Background: β -Cell-specific surface targets are required for noninvasive monitoring of β -cell mass, which could be used for evaluation of new diabetes treatments as well as to help unravel pathogenic mechanisms underlying β -cell dysfunction.

Methods and Results: Antibody-based proteomics was used to identify and explore a set of islet cell-specific proteins. A search algorithm in the Human Protein Atlas was performed to identify islet-specific proteins. This led to the identification of 27 hits, of which 12 showed a clear membranous expression pattern or had predicted transmembrane regions. The specificity of the identified proteins was investigated by immunohistochemical staining of pancreas sections from diabetic and nondiabetic subjects. None of these antigens were expressed in the exocrine pancreas. Colocalization with insulin and glucagon was further determined by confocal microscopy using isolated human islets. All antibodies specifically stained human islets and colocalization analysis revealed that four proteins were exclusively expressed in β -cells. Of note, these antibodies were negative in sections from subjects with long-standing T1D.

Conclusions: The present study identified four specific β -cell proteins: DGCR2, GBF1, GPR44 and SerpinB10, the expression of which has not previously been described.

The identification of specific β -cell markers, which could allow the assessment of β -cells in a noninvasive manner, is of paramount importance in terms of diagnostic and therapeutic approaches. Up to now, several molecules have been tested as possible targets for β -cell assessment, without finding an ideal marker [1]. The best β -cell marker should be membrane-expressed and highly represented in order to allow discerning β -cells from the more abundant cells characterizing the exocrine pancreas. A surface β -cell-specific marker could be used as a target for tracers for imaging technique, or even as a target for interventions aiming at preserving β -cell mass/function or to monitor the effect of therapies on β -cells.

Antibody-based proteomics is an emerging approach which provides a strategy to explore protein expression patterns on a genome-wide scale [2]. The application of this approach is possible thanks to the Human Protein Atlas (HPA) project which has generated antibodies towards at least one major isoform of all human proteins encoded by the human genome [3]. These antibodies are then used to map protein expression in specific tissues under normal or pathological conditions.

In the present study, the antibody-based proteomic approach allowed the identification of four specific proteins: DGCR2, GBF1, GPR44 and SerpinB10, which all showed β -cell-specific immunoreactivity mainly localized to the plasma membrane in islets. This positivity was found in nondiabetic subjects and in those with diabetes and insulin-positive cells, but not in patients with diabetes and insulin-negative cells.

These results are of note and represent a step forwards in the process of a better characterization of β -cells.

Glycotoxin and autoantibodies are additive environmentally determined predictors of type 1 diabetes: a twin and population study

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Diabetes 2012;61:1192–1198

Background: In T1D, diabetes-associated autoantibodies, including islet cell antibodies (ICAs), reflect adaptive immunity, while increased serum N^ε-carboxymethyl-lysine (CML), an advanced glycation end product (AGE), is associated with proinflammation. The aim of this study was to investigate whether serum CML and autoantibodies predicted T1D and to what extent they were determined by genetic or environmental factors.

Methods: Of 7,287 unselected screened schoolchildren, 115 were ICA+ and were tested for baseline CML and diabetes autoantibodies and followed (for median 7 years), whereas a random selection (n = 2,102) had CML tested. CML and diabetes autoantibodies were determined in twin pairs discordant for T1D (32 monozygotic, 32 dizygotic pairs).

Results: CML was increased in ICA+ and prediabetic schoolchildren and in diabetic and nondiabetic twins (all $p < 0.001$). Elevated levels of CML in ICA+ children were a persistent, independent predictor of diabetes progression, in addition to autoantibodies and HLA risk. In a twin model, shared familial environment explained 75% of CML variance, and nonshared environment explained all autoantibody variance.

Conclusions: Serum CML, a glycotoxin, emerged as an environmentally determined diabetes risk factor, in addition to autoimmunity and HLA genetic risk, and a potential therapeutic target.

In search for predictive markers of T1D, the present study assessed the predictive role of an AGE, such as CML, and autoantibodies. Starting with a population-based study, where autoantibody-positive children (ICA+, n 115) were identified and followed for diabetes development, one of the main findings was that not only autoantibodies, but also CML levels, were significantly increased in subjects who went into develop T1D. CML emerged to be an important predictor of T1D, in addition to autoantibodies and HLA predisposition. In addition, it was shown that environmental factors significantly contributed to the variability of both CML and autoantibodies. Interestingly, whereas shared (familial) environmental factors were an important determinant for CML, nonshared environmental factors mainly influenced autoantibodies.

This study adds a further detail to the 'AGE-T1D relationship'. From an original interest in AGEs mainly in relation to the development of vascular complications of diabetes, the focus is now on the role of AGEs in the pathogenesis of T1D. The next step is to understand the mechanisms responsible for increased AGEs, in order to identify a potential target for preventive and therapeutic strategies. Diet is a well-known important source of AGEs. Therefore, early life modifications in diet could represent a potential preventive strategy for T1D.

Mixed chimerism and growth factors augment β -cell regeneration and reverse late-stage type 1 diabetes

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Sci Transl Med 2012;4:133ra59

Background: Islet transplantation is the only curative therapy for late-stage T1D, but the beneficial effect is limited in its duration, due to the effects of both auto- and alloimmunity. Another important limitation is the shortage of donor islets. The present study tested the effect of combining mixed chimerism with administration of gastrin and epidermal growth factor (EGF). These two strategies are known to be effective when applied individually in new onset by not late stages T1D.

Methods: Induction of mixed chimerism was associated with administration of gastrin and EGF in non-obese diabetic (NOD) mouse model of T1D.

Results: Combination therapy of induced mixed chimerism under a radiation-free nontoxic anti-CD3/CD8 conditioning regimen and administration of gastrin/EGF increased both β -cell neogenesis and replication, resulting in reversal of late-stage T1D in NOD mice.

Conclusions: If successfully translated into humans, this combination therapy could replace islet transplantation as a long-term curative therapy for T1D.

Improvement of islet function in a bioartificial pancreas by enhanced oxygen supply and growth hormone-releasing hormone agonist

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Proc Natl Acad Sci USA 2012;109:5022–5027

Background: Islet transplantation is a feasible therapeutic alternative for metabolically labile patients with T1D. However, the application of this methodology is still limited by several factors: shortage of donor organs, gradual loss in graft function over time, and chronic need for immunosuppression. In the present study a promising approach to address some of the limitations of islet transplantation was tested.

Methods: A macrochamber specially engineered for islet transplantation was implanted in streptozotocin-induced diabetic rats, in association with pretreatment of islet cells with the growth hormone-releasing hormone (GHRH) agonist JI-36.

Results: A subcutaneous implantable device was created allowing for controlled and adequate oxygen supply and providing immunological protection of donor islets against the host immune system. Pretreatment with the GHRH agonist, JI-36, significantly enhanced graft function by improving glucose tolerance and increasing β -cell insulin reserve and allowed a reduction in the transplanted islet mass required to achieve metabolic control. As a result of hypervascularization of the tissue surrounding the device, no relevant delay in insulin response to glucose changes was observed.

Conclusions: This system opens up a fundamental strategy for therapy of diabetes and may provide a promising avenue for future approaches to xenotransplantation.

Islet transplantation is considered the definitive cure for T1D, through the reconstitution of the pool of β -cells, destroyed by the autoimmune attack. The original very promising results of the Edmonton Protocol generated tremendous excitement over the application of pancreatic islet transplantation as a viable approach to achieve consistent insulin independence in patients with T1D [4]. However, soon after those initial highly promising results, it became clear that this is a treatment still far away from a potential wide application in patients with T1D. One of the main obstacles to overcome is finding 'islet donors'. In addition, it has become evident that islet transplants last only

about 3 years. Several factors influence the success of this method, including the need of islets from more than one donor, prolonged immunosuppression therapies, the low rate of survival of transplanted islets, due to the different characteristics of the environment where they are implanted compared to the natural milieu in the pancreas. Therefore, there is ongoing research to overcome these limitations.

In this context, Wang et al. assessed a combination of radiation-free conditioning regimen to induce mixed chimerism associated with administration of growth factors, such as gastrin and EGF, to reverse late stage T1D in nonobese diabetic mice. Through a series of elegant experiments the authors clearly showed that the 'combination of two treatments is definitively better than one'. In animal models, mixed chimerism had already emerged as a valid approach to reverse autoimmunity, and eliminate insulinitis. However, those benefits occur in cases of new-onset diabetes but not in late cases. Similarly, gastrin/EGF can stimulate β -cell regeneration in animal models with new-onset diabetes but not in late-stage T1D. Combination therapy was effective in reversing late-stage diabetes and improved insulin sensitivity, highlighting that during late stages of diabetes it is important not only to control autoimmunity, but also to promote regeneration of β -cells, whose number is very limited at that stage.

Another exciting finding comes from the study by Ludwig et al., where the authors tried to improve oxygenation of encapsulated islets in order to increase their survival. Encapsulated islets are generally used to avoid the attack by the immune system. However, this process can limit oxygenation of transplanted islets and therefore contribute to cell death. In the present study an implantable subcutaneous bioartificial chamber system, specifically created for encapsulated islets, was developed. This system, which is designed for subcutaneous implantation, is minimally invasive and easily retrievable, and provided a good level of oxygenation of the islets, while creating an immune-barrier sufficient to prevent allogeneic immune response. Successful survival of transplanted islets was improved by conditioning of pancreatic islets with a GHRH agonist, given the high expression of receptors for this factor on pancreatic cells. This pretreatment reduced cells apoptosis and preserved β -cell proliferative capacity.

Although both treatments are based on animal studies, they are promising and represent tremendous progresses in the field of islet transplantation.

New concerns 1

Vitamin D and T1D: the prenatal origin of an association

Maternal serum levels of 25-hydroxyvitamin D during pregnancy and risk of type 1 diabetes in the offspring

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Diabetes 2012;61:175–178

Background: Risk of T1D has been reported to be reduced after intake of vitamin D supplements during pregnancy or early childhood. The aim of this study was to assess whether lower maternal serum concentrations of 25-hydroxyvitamin D (25-OH-D) during pregnancy were associated with an increased risk of T1D in the offspring.

Methods: 25-OH-D levels were measured using a radioimmunoassay on samples from late pregnancy in 109 women delivering a child who developed T1D before 15 years of age (case subjects) and from 219 control women.

Results: There was a trend toward a higher risk of T1D with lower levels of 25-OH-D during pregnancy: the odds of T1D being 2.38 (1.12–5.07) for the offspring of women with the lowest levels of 25-OH-D (first quartile) compared to the offspring of women with levels in the highest quartile.

Conclusion: Reduced maternal 25-OH-D levels increase the risk of developing T1D in the offspring. If these results are confirmed, they could provide support for the initiation of a randomized intervention trial to prevent T1D in children by enhancing maternal 25-OH-D levels during pregnancy.

Over the last years there has been a growing interest in the immunomodulatory effect of vitamin D and in its association with the pathogenesis of autoimmune diseases, such as T1D [5]. Reduced levels of vitamin D have been reported in children and adults with T1D compared to healthy controls. In addition, there have also been studies assessing the potential role of polymorphisms in genes implicated in vitamin D metabolism in the pathogenesis of T1D [6].

This recent study reports an interesting association between reduced maternal serum vitamin D levels and risk of developing T1D in the offspring before the age of 15 years. Levels of vitamin D were reduced in mothers of offspring who developed T1D compared to controls (65.8 vs. 73.1 nmol/l). Of particular note was the finding that children born from women with a 25-OH-D level in the lowest quartile had a risk of developing T1D more than twofold higher than those born from women with a 25-OH-D level in the highest quartile. These findings suggest that vitamin D could influence T1D risk already during prenatal life, by influencing the immune system. In fact, although the exact mechanisms linking reduced vitamin D levels to T1D are not yet completely understood, there is evidence suggesting that vitamin D can enhance immunologic tolerance [7].

These results are of interest given that they provide further support for the potential role of vitamin D in the pathogenesis of T1D and, in the meantime, they confirm the role of environmental factors acting early in life in the pathogenesis of this autoimmune disease. If these results are confirmed by future studies, they could represent the driving force for trials with vitamin D supplementation during pregnancy as a preventive strategy for T1D.

New concerns 2

Secular changes in T1D immunophenotype

Rising incidence of type 1 diabetes is associated with altered immunophenotype at diagnosis

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Diabetes 2012;61:683–686

Background: Over the last decades there has been a significant increase in the incidence of T1D in children. The aim of this study was to assess whether this trend is associated with changes in the patterns of humoral islet autoimmunity at diagnosis.

Methods: Autoantibodies to insulin (IAA), GAD (GADA), islet antigen-2 (IA-2A), and zinc transporter 8 (ZnT8A) were measured by radioimmunoassay in sera collected from children and young adults with newly diagnosed T1D between 1985 and 2002. The effect of date at diagnosis on prevalence and levels of these autoantibodies was assessed, with adjustments for age and HLA class II genetic risk.

Results: Over the study period there was a significant increase in the prevalence of IA-2A and ZnT8A together with increased levels of IA-2A, ZnT8A, and IA-2 β autoantibodies. In contrast, no significant changes occurred in the prevalence and levels of IAA and GADA.

Conclusions: The increased prevalence and levels of IA-2A, ZnT8A, and IA-2 β at diagnosis suggest that the process leading to T1D is now characterized by a more intense humoral autoimmune response.

The increasing incidence in childhood-onset T1D, particularly in younger children, is a worldwide established phenomenon. Changes in environmental factors have been claimed as the major determinant of the rise in T1D cases. Interestingly, there have been observations suggesting that the increasing incidence in T1D is associated with a reduced frequency of high-risk HLA-class II genotypes and a higher prevalence of low-risk genotypes [8].

The intriguing hypothesis tested in the present study was that the rising incidence in T1D could be associated with an altered immunophenotype at diagnosis. Indeed, this was the case, with a detected higher prevalence of autoantibodies, such as IA-2 and ZnT8A, as well as higher levels of these autoantibodies, over time, from 1985 to 2002. These autoantibodies are known to appear after GADA and IAA, whose levels and prevalence did not significantly change over time in this study. This finding along with the increased levels of IA-2 β A, among IA-2A-positive patients, suggest a more intense and mature humoral

autoimmunity in patients diagnosed during more recent years, which might lead to faster development of the disease in at-risk individuals. The association between date at diagnosis and changes in the immunophenotype was independent of age and HLA genotype. These results suggest the potential effect of changes in the environment and/or lifestyle on the humoral autoimmune response to islet antigens. Identifying the mechanisms underlining this link would help in understanding the rising trend in T1D incidence, and hopefully help in developing new potential preventive strategies.

Important for clinical practice 1

New important result from the landmark DCCT/EDIC studies

Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes

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N Engl J Med 2011;365:2366–2376

Background: An impaired glomerular filtration rate (GFR) leads to end-stage renal disease and increases the risks of cardiovascular disease and death. This study tested the effects of intensive insulin therapy on the development of impaired GFR.

Methods: The Diabetes Control and Complications Trial (DCCT) recruited 1,441 persons with T1D who were randomized to 6.5 years of intensive diabetes therapy or to conventional diabetes therapy. Subsequently, 1,375 participants were followed in the observational Epidemiology of Diabetes Interventions and Complications (EDIC) study. Serum creatinine levels were measured annually during the DCCT and EDIC studies. GFR was estimated with the use of the Chronic Kidney Disease Epidemiology Collaboration formula. Incident impaired GFR was defined as an estimated GFR of <60 ml/min/1.73 m² of body surface area at two consecutive study visits.

Results: Over a median follow-up period of 22 years, impaired GFR developed in 24 participants assigned to intensive therapy (IT) and in 46 assigned to conventional therapy (CT) (risk reduction with IT, 50% (95% CI 18–69); $p = 0.006$). End-stage renal disease developed in 8 participants in the IT group and in 16 in the CT group. IT was associated with a reduction in the mean estimated GFR of 1.7 ml/min/1.73 m² during the DCCT study compared to CT, but during the EDIC study was associated with a slower rate of reduction in the GFR and 2.5 ml/min/1.73 m² higher mean estimated GFR compared to CT ($p < 0.001$ for both comparisons). Adjustments for HbA_{1c} or albumin excretion rate completely attenuated the beneficial effect of IT on GFR.

Conclusions: Intensive insulin therapy started early in the course of T1D and when compared with conventional diabetes therapy was associated with a significantly lower risk of impaired GFR.

Impaired GFR is an important risk factor for the progression to end-stage renal disease and for cardiovascular morbidity and mortality in patients with T1D [9]. Over previous years, the DCCT and EDIC studies have provided crucial evidence on the many benefits of good glycemic control, as achieved with an intensive insulin therapy, started early in the course of diabetes, in reducing risk for the development and progression of albuminuria, diabetic retinopathy, neuropathy as well as cardiovascular disease. This publication further adds to those previous findings, by assessing another important endpoint: GFR. Perturbations in GFR are common in patients with T1D, where during early stages hyperfiltration can be the main finding [9], whereas from, or even slightly before, the development of microalbuminuria, there is a progressive decrease in GFR, which can prelude to the stage of renal failure.

Epidemiological studies have shown the adverse prognostic effect of impaired GFR, although until now there has been a lack of long-term longitudinal studies assessing the effect of intensive insulin therapy on GFR. The DCCT/EDIC studies offer a unique source of data with up to 22 years of follow-up. Risk of impaired GFR was reduced by 50% in patients in the intensive treatment group. Similarly, cases of end-stage renal disease, although low in numbers, tended to be lower in the intensive treatment group. The differences in GFR between the two groups were mainly explained by differences in HbA_{1c} and albuminuria. These data reiterate the importance of a strict glycemic control started early

in the course of diabetes to prevent renal complications. The DCCT/EDIC data mainly explored the effect of intensive insulin therapy without assessing whether other treatment strategies with ACE inhibitors or other agents could have an additional beneficial effect on GFR. Further studies are required to test that.

Important for clinical practice 2

Feasibility of a behavioral intervention for T1D

Clinic-integrated behavioral intervention for families of youth with type 1 diabetes: randomized clinical trial

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Pediatrics 2012;129:e866–873

Background: The aim of this study was to test the effect of a low-intensity, clinic-integrated behavioral intervention for families of youth with T1D, on diabetes management outcomes.

Methods: A 2-year randomized clinical trial of a clinic-integrated behavioral intervention was performed and included 390 families of youth with T1D. The primary outcome was HbA_{1c}, which was measured at each clinic visit and assessed centrally. At each visit, blood glucose meter data were downloaded. In addition, treatment adherence was assessed by using a semistructured interview at baseline, mid-study, and follow-up.

Results: The behavioral intervention was associated with a significant improvement in glycemic control from baseline to 24 months ($p = 0.03$). A significant intervention by age interaction ($p < 0.001$) was detected such that whereas among participants aged 12–14 a significant effect on glycemic control was observed ($p = 0.009$ for change from baseline to 24 months; $p = 0.035$ for mixed-effect model across study duration), no effect was found in youth aged 9–11 years. The intervention did not have any significant effect on child or parent reports of adherence.

Conclusions: The low-intensity clinic-integrated behavioral intervention for families of youth with T1D was effective in preventing the deterioration in glycemic control evident during adolescence, and it might represent a model for integrating medical and behavioral sciences in clinical care.

Diabetes management in youth can be challenging, particularly during adolescence. Diabetes treatment is based on the integration of medical therapies, nutrition as well as psychological and behavioral support for children and their families.

Previous studies had already shown the positive effect of behavioral interventions in improving diabetes management. These interventions include greater parental involvement, monitoring responsibility-sharing, lower family conflicts, greater coping and problem-solving skills. However, an important barrier is the translation of behavioral interventions into standard clinical practice. This requires that behavioral interventions are indeed effective, but also simple and feasible.

The present multicenter study was performed on 390 families of youth with T1D and an age range between 9 and 14.9 years. The behavioral intervention, called WE-CAN (Working together, Exploring barriers, Choosing solutions, Acting on our plan, Noting results), was designed to help families improve diabetes management, and its cardinal feature was that it was delivered to both parents and children. The key points on which it was based were: facilitating problem-solving, communication skills, and responsibility sharing. Specially trained personnel were responsible for the different parts of the intervention program, including pre-visit telephone contact, in-person contact during clinic visits, and follow-up telephone calls. Each session lasted about 30 min and feedback about the sessions was collected by phone in the weeks following clinic visits.

This behavioral intervention was successful in improving glycemic control over the study period. The effect was particularly more evident in adolescents than in younger children. This is an important finding given that management of diabetes is particularly complex during adolescence, when dealing with a chronic condition, such as T1D, needs to fit within the complex hormonal,

metabolic and psychological changes of this phase of life. In addition, the beneficial effect observed mainly in adolescents suggests that, as previously shown, intervention strategies can be particularly effective when youth start taking greater responsibility for their diabetes management.

The take-home message of this study is the utility of incorporating a low-intensity behavioral intervention into clinical care in order to improve glycemic control, particularly in adolescents.

Clinical trials, new treatments 1

New insulin on the horizon

Insulin degludec, an ultralong-acting basal insulin, versus insulin glargine in basal-bolus treatment with mealtime insulin aspart in type 1 diabetes (BEGIN basal-bolus type 1): a phase 3, randomized, open-label, treat-to-target noninferiority trial

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Lancet 2012;379:1489–1497

Background: In patients with T1D, intensive basal-bolus insulin therapy can improve glycemic control and reduce the risk of long-term complications. Insulin degludec is a new ultralong-acting basal insulin. The present study aimed to compare the efficacy and safety of insulin degludec and insulin glargine, both administered once daily with mealtime insulin aspart, in basal-bolus therapy for T1D.

Methods: An open-label, treat-to-target, noninferiority trial, was undertaken at 79 sites in six countries, and recruited adults (aged ≥ 18 years) with T1D ($HbA_{1c} \leq 10\%$ (86 mmol/mol)) and who had been treated with basal-bolus insulin for at least 1 year. Participants were randomly assigned in a 3:1 ratio, with a computer-generated blocked allocation sequence, to insulin degludec or insulin glargine. The primary outcome was noninferiority of degludec to glargine, assessed as a reduction in HbA_{1c} after 52 weeks, with the intention-to-treat analysis.

Results: Of 629 participants, 472 were randomly assigned to insulin degludec and 157 to insulin glargine; all were analyzed in their respective treatment groups. After 1 year of treatment, HbA_{1c} declined by 0.40% points (SE 0.03) with insulin degludec and 0.39% points (0.07) with insulin glargine (estimated treatment difference -0.01% points (95% CI -0.14 to 0.11); $p < 0.0001$ for noninferiority testing). A similar number of participants (40 vs. 43%) achieved a target HbA_{1c} below 7% (< 53 mmol/mol). Rates of overall confirmed hypoglycemia (plasma glucose < 3.1 mmol/l or severe) were similar in insulin degludec and insulin glargine groups (42.54 vs. 40.18 episodes per patient-year of exposure). The rate of nocturnal confirmed hypoglycemia was 25% lower with degludec than with glargine (4.41 vs. 5.86 episodes per patient-year of exposure; 0.75 (0.59–0.96); $p = 0.021$). Occurrence of serious adverse events (14 vs. 16 events per 100 patient-years of exposure) was similar for insulin degludec and insulin glargine groups.

Conclusions: Insulin degludec appears to be a useful basal insulin for patients with T1D, providing effective glycemic control and reducing the risk of nocturnal hypoglycemia.

Basal-bolus insulin regimen is the standard approach for patients with T1D. However, this approach is far from perfect in mimicking the physiological endogenous insulin pattern.

A new insulin analogue is now on the horizon and phase 2 trials have already shown promising results. Insulin degludec is a new basal insulin with an amino acid sequence identical to human insulin except for removal of threonine at B30. At B29, a glutamic acid spacer is attached that bridges to a 16-carbon diacid. This new insulin forms soluble multihexamer assemblies after subcutaneous injection, resulting in an ultralong action profile, with a reported half-life > 25 h and activity > 40 h. These characteristics reduce plasma concentrations and variability and create a flat steady-state profile following a once-daily injection.

In this recent phase 3 trial, designed as a 52-week randomized, controlled, open-label, treat-to-target noninferiority trial, insulin degludec, in combination with prandial insulin aspart, led to similar glycaemic control as insulin glargine, but the main study finding was a 25% reduction in nocturnal hypoglycemia associated with insulin degludec. This finding is of note and likely reflects the flatter profile of insulin degludec than glargine.

Nocturnal hypoglycemia is associated with poor quality of sleep, decreased sense of wellbeing, reduced productivity and fatigue. Therefore, reducing nocturnal hypoglycemia could significantly improve quality of life. Asymptomatic episodes of hypoglycemia are very common in children and adolescents, in whom they can reduce counterregulation and thereby increase the risk of subsequent prolonged and severe hypoglycemia, associated occasionally with coma. Therefore, there is a great interest in testing insulin degludec in this age group in order to improve their glycaemic control while avoiding episodes of hypoglycemia.

Clinical trials, new treatments 2

Disappointing results from clinical trials

GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus

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N Engl J Med 2012;366:433–442

Background: The 65-kDa isoform of glutamic acid decarboxylase (GAD65) is a major autoantigen in T1D. This study assessed whether treatment with alum-formulated GAD65 (GAD-alum) can preserve β -cell function in patients with recent-onset T1D.

Methods: The study population included 334 patients with T1D aged between 10 and 20 years, with fasting C-peptide levels >0.3 ng/ml (0.1 nmol/l), and detectable serum GAD65 autoantibodies. Within 3 months after diagnosis, patients were randomly assigned to receive one of three study treatments: four doses of GAD-alum, two doses of GAD-alum followed by two doses of placebo, or four doses of placebo. The primary study outcome was the change in the stimulated serum C-peptide level between the baseline and the 15-month visit. Secondary outcomes were HbA_{1c} level, mean daily insulin dose, rate of hypoglycemia, and fasting and maximum stimulated C-peptide levels.

Results: A similar decline in stimulated C-peptide levels occurred in all the study groups and there was no significant difference in the primary outcome at 15 months between the combined active drug groups and the placebo group ($p = 0.10$). In addition, there were no differences in insulin dose, HbA_{1c}, hypoglycemic rates between patients treated with GAD-alum and those receiving placebo. Infrequent and mild adverse events occurred with similar rates in the three groups.

Conclusions: Treatment with GAD-alum did not significantly reduce the loss of stimulated C peptide or improve clinical outcomes over a 15-month period in newly diagnosed patients with T1D.

Disappointing results have emerged from the first phase 3 immune intervention trials in T1D, which are partly unexpected, given that phase II trials showed promising results [10].

In the study of Ludvigsson et al., GAD-alum treatment failed to preserve β -cell secretion over time as well as to improve glycaemic control. These results were completely different from those from a previous phase II study, where treatment with GAD-alum was associated with a preserved C-peptide at 30 months and preserved stimulated C-peptide at 15 months.

The authors suggest that differences in populations or the larger number of clinicians, with potential different approaches to conventional treatments, involved in the phase III trial could explain these discordant results. Another hypothesis is that the differences in age between the treatment groups, including more subjects aged 10–11 years in the GAD-alum group, could have influenced the study results. In fact, it is well known that C-peptide tends to decline faster in younger subjects. Differences in the time of year (seasonal effect) when treatment was implemented might be another confound-

ing factor, as suggested by the finding of better outcomes in those treated during March–April (when all the phase II subjects had been treated). In addition, during the GAD-alum phase III trial, an influenza epidemic occurred, with an extensive vaccination program, and these phenomena could have influenced the trial results. Overall, this study highlights some important factors to be taken into account in future studies: (1) a better patient stratification might be implemented; (2) longer treatment periods might be required, and (3) more needs to be learnt on the mechanisms implicated in the pathogenesis of T1D in order to implement better treatment strategies. In addition, the lack of success of GAD-alum as well as of other immunotherapy suggests that, like in other complex diseases, more than one intervention directed towards different targets might be needed.

New Mechanisms

Huntingtin-interacting protein 14 is a type 1 diabetes candidate protein regulating insulin secretion and β -cell apoptosis

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Proc Natl Acad Sci USA 2011;108:E681–688

Background: T1D is a complex disease with a strong genetic component, and several loci are known to increase T1D susceptibility risk, although only few causal genes have currently been identified. To identify disease-causing genes in T1D, an in-silico ‘phenome-interactome analysis’ on a genome-wide linkage scan dataset was performed.

Methods: A phenome-interactome network analysis on a published T1D genome-wide linkage study revealed 11 genomic regions with linkage to T1D.

Results: A total of 11 genes were predicted to be likely disease genes in T1D, including the INS gene. An unexpected top-scoring candidate gene was huntingtin-interacting protein (HIP)-14/ZDHHC17. Immunohistochemical analysis of pancreatic sections showed that HIP14 is almost exclusively expressed in insulin-positive cells in islets of Langerhans. RNAi knockdown experiments established that HIP14 is an antiapoptotic protein required for β -cell survival and glucose-stimulated insulin secretion. Proinflammatory cytokines, such as IL-1 β and IFN- γ , downregulated HIP14 expression in insulin-secreting INS-1 cells and in isolated rat and human islets. Overexpression of HIP14 was associated with a decrease in IL-1 β -induced NF- κ B activity and protection against IL-1 β -mediated apoptosis.

Conclusions: This study highlights that network biology is a valid approach to identify important genes for T1D that represent the basis for more specific and targeted therapeutic approaches.

Genome-wide association studies (GWAS) have identified many genetic variants associated with complex diseases. More than 40 genetic regions have been significantly associated with T1D, however it is not clear which are the causative variants and the specific genes in these regions. The study from Berchtold et al. is an example of the ‘post-GWAS’ era, when GWAS data are being implemented with other techniques in order to understand the role and function of identified loci.

Using integrative bioinformatics, based on an in-silico ‘phenome-interactome network analysis’ approach, 11 T1D candidate proteins were identified, including insulin. However, the new finding was the identification of the huntingtin-interacting protein (HIP)-14 protein as a new candidate protein for T1D. This protein emerged to be expressed in β -cells and to play a key role for β -cell survival. Reduced levels of HIP-14 or knockout of HIP-14 in animal models was associated with increased apoptosis of β -cells. Downregulation of HIP-14 emerged to be the potential mechanism mediating cytokine-induced β -cell apoptosis in T1D. Overall, this study demonstrates the value and feasibility of a new approach based on the systematic integration of genetic information with protein-protein interaction data to identify proteins which could become targets for preventive and therapeutic interventions.

Identification of novel type 1 diabetes candidate genes by integrating genome-wide association data, protein-protein interactions, and human pancreatic islet gene expression

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Diabetes 2012;61:954–962

Background: Genome-wide association studies (GWAS) have allowed the discovery of more than 40 susceptibility loci for T1D. However, GWAS do not lead to identification of the gene or genes in a given locus associated with disease, and they do not typically give any information on the broader context in which the disease genes operate.

Methods: T1D GWAS data were integrated with protein-protein interactions to construct biological networks of relevance for disease. Expressional profiling in human pancreatic islets exposed to proinflammatory cytokines was performed. Confirmation of new candidate genes was then performed in insulin-secreting INS-1 β -cells.

Results: A total of 17 networks were identified. Three networks were significantly enriched for cytokine-regulated genes and thus likely to play an important role for T1D in pancreatic islets. Eight of the regulated genes (CD83, IFNGR1, IL17RD, TRAF3IP2, IL27RA, PLCG2, MYO1B, and CXCR7) in these networks also contained single nucleotide polymorphisms nominally associated with T1D. The expression and cytokine regulation of these new candidate genes were confirmed in insulin-secreting INS-1 β -cells.

Conclusions: These data provide novel insight on the T1D pathogenesis and thus may provide the basis for the design of novel treatment strategies.

In line with the other study by Berchtold et al., which led to the identification of HIP-14 as being associated with T1D, through a combination of genetic data with protein-protein interaction data, this study used extended GWAS data in order to characterize the role of previously identified T1D loci. Network- or pathway-based approaches have been applied to extend GWAS in other diseases, and they are considered as the next step in the process of GWAS data mining. In the present study, GWAS data were integrated with protein-protein interactions and gene expression in β -cells in order to facilitate a systems-based understanding of the mechanisms implicated in the pathogenesis of T1D. This approach led to the identification of an initial number of 17 networks. Among them, 3 were particularly enriched for genes affected by proinflammatory cytokines in the human islets and included 8 genes containing SNPs associated with T1D. Further studies using similar approaches will further clarify mechanisms implicated in the complex pathogenesis of T1D.

Identification of type 1 diabetes-associated DNA methylation variable positions that precede disease diagnosis

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PLoS Genet 2011;7:e1002300

Background: Monozygotic (MZ) twin pair discordance for childhood-onset T1D is approximately 50%, implicating roles for both genetic and nongenetic factors in the etiology of the disease. The study aim was to test whether epigenetic variation could underlie some of the nongenetic components of T1D etiology.

Methods: An epigenome-wide association study (EWAS) for T1D was performed. Genome-wide DNA methylation profiles of purified CD14⁺ monocytes (an immune effector cell type relevant to T1D pathogenesis) from 15 T1D-discordant MZ twin pairs were generated.

Results: The EWAS identified 132 different CpG sites, at which intra-MZ pair DNA methylation differences significantly, correlated with the diabetic state, i.e. T1D-associated methylation variable positions (T1D-MVPs). An independent set of T1D-discordant MZ pairs was used to confirm that the T1D-MVPs display statistically significant intra-MZ pair DNA methylation differences in the expected direc-

tion ($p = 0.035$). In order to establish the temporal origins of the T1D-MVPs, two further EWAS datasets were generated and they showed that, when compared with controls, T1D-MVPs are enriched in singletons both before ($p = 0.001$) and at ($p = 0.015$) disease diagnosis, and also in singletons positive for diabetes-associated autoantibodies but disease-free even after 12 years of follow-up ($p = 0.0023$).

Conclusions: T1D-MVPs appear to arise very early in the etiological process that leads to overt T1D. This EWAS of T1D represents an important contribution toward understanding the etiological role of epigenetic variation in T1D.

Epigenetic modifications represent important changes which are implicated in physiological processes, such as regulation of gene transcription and maintenance of genome integrity [11]. A contribution of epigenetic modifications has also been shown in several human diseases, and these changes can occur early in life.

Monozygotic twins represent a valuable model to dissect the relative contributions of the genetic background from environmental influences in human diseases, and thus to assess the potential contribution of epigenetic changes in the pathogenesis of T1D.

In the present study, 15 T1D discordant MZ twin pairs were recruited to perform an epigenome-wide association study, which revealed the presence of T1D-specific methylation positions, defined T1D-MVPs, in the coaffected cotwins. Interestingly, further analysis in other sets of twins highlighted that some of the identified T1D-MVPs are present both before and after the onset of T1D and even in individuals positive for autoantibodies, but who have not developed diabetes yet. These results suggest that these T1D-MVPs could have a role in the pathogenesis of T1D and not only reflect a marker of the disease, due to their temporal appearance before the onset of T1D. Further studies need to assess whether these changes occur even before the appearance of autoantibodies in order to further clarify their pathogenic role in T1D, and the stage when they act. This study represents an important step forwards in understanding the pathogenesis of T1D and it is a good example of a well-designed epigenetic study, which allowed assessment of the temporal origin of T1D-associated epigenetic variations.

Cesarean section and interferon-induced helicase gene polymorphisms combine to increase childhood type 1 diabetes risk

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Diabetes 2011;60:3300–3306

Background: The aim of the study was to investigate the association between cesarean delivery, islet autoimmunity and T1D, and genes involved in T1D susceptibility.

Methods: Cesarean section was examined as a risk factor in 1,650 children born to a parent with T1D and followed from birth for the development of islet autoantibodies and T1D.

Results: Children delivered by cesarean section ($n = 495$) had a more than twofold higher risk for T1D than children born by vaginal delivery (hazard ratio (HR) 2.5 (95% CI 1.4–4.3); $p = 0.001$, adjusted HR 2.7; 1.5–5.0; $p = 0.001$). Cesarean section did not increase the risk for islet autoantibodies ($p = 0.6$) but was associated with a faster progression to diabetes after the appearance of autoimmunity ($p = 0.015$). The reported association was detected in children with and without high-risk HLA genotypes. In addition, cesarean section appeared to interact with immune response genes, including CD25 and the interferon-induced helicase 1 gene (IFIH1). With regard to IFIH1 genotypes, an increased risk for T1D was only seen in children who were delivered by cesarean section and had T1D-susceptible IFIH1 genotypes (12-year risk 9.1 vs. <3% for all other combinations; $p < 0.0001$).

Conclusions: The present study suggests that T1D risk modification by cesarean section may be linked to viral responses in the preclinical autoantibody-positive disease phase.

The pathogenesis of T1D is well established to be multifactorial with a contribution of genetic and environmental factors along with their interaction.

Several environmental factors have been investigated. Interestingly, cesarean section has been one of these factors, mainly due to the observation that in parallel with the increasing incidence of T1D, there has been an increasing number of children delivered by cesarean section. A recent meta-analysis including 20 retrospective studies highlighted a 20% increased risk of T1D in children delivered by

cesarean section [12]. This association has been explained by the finding that children delivered by cesarean section have altered gut microbiotic composition and immune response. In particular, it seems that the types of bacterial found on the newborn skin are different in those delivered by cesarean as compared to those born by vaginal delivery [13]. This in turn could influence immune responses and susceptibility to autoimmune diseases.

This recent study is based on follow-up of children recruited to the BABYDIAB study, a longitudinal prospective study which recruited around 1,650 children born to a parent with T1D between 1989 and 2000, and has been following them since. Autoantibodies have been measured during follow-up and new cases of diabetes recorded. The risk of developing T1D by age 12 in this cohort was higher in children delivered by cesarean section than in those born by vaginal delivery (4.8 vs. 2.8%). A further assessment was the potential interaction between cesarean section, as an environmental determinant, with the genetic background. Genotypes of four genetic regions, known to be associated with T1D and with the immune response, were assessed: IF1H1, CD25, PTPN22 and HLA class II genes. Interaction was found for CD25 and IF1H1. Interestingly for IF1H1, only children with the combination of the risk genotype and delivered by cesarean section were at higher risk of developing T1D. IF1H1 is known to induce the secretion of immune mediators in response to viral infection, mediators which generally block viral replication and can stimulate expression of MHC complex proteins, which can promote destruction of β -cells.

This study sheds more light on the complex pathogenesis of T1D, mainly highlighting the importance of considering the interaction between environmental factors and the individual genetic background when considering disease risk.

New Genes

The human pancreatic islet transcriptome: expression of candidate genes for type 1 diabetes and the impact of proinflammatory cytokines

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PLoS Genet 2012;8:e1002552

Background: T1D is an autoimmune disease in which pancreatic β -cells are destroyed by infiltrating immune cells and by cytokines released by these cells. Signaling events occurring in the pancreatic β -cells are decisive for their survival or death in diabetes.

Methods: RNA sequencing was used to identify transcripts, including splice variants, expressed in human islets of Langerhans under control conditions or following exposure to the proinflammatory cytokines interleukin-1 β (IL-1 β) and interferon- γ (IFN- γ). In addition, it was assessed whether putative candidate genes for T1D, previously identified by GWAS, are expressed in human islets.

Results: A total of 29,776 transcripts were identified as expressed in human islets. Expression of around 20% of these transcripts was modified by proinflammatory cytokines, including apoptosis- and inflammation-related genes. Chemokines were among the transcripts most modified by cytokines. 35% of the genes expressed in human islets undergo alternative splicing, and cytokines caused substantial changes in spliced transcripts. 25/41 of the candidate genes for T1D were expressed in islets, and cytokines modified expression of several of these transcripts.

Conclusions: The present study doubles the number of known genes expressed in human islets and shows that cytokines modify alternative splicing in human islet cells. A large number of cytokines and chemokines are expressed in β -cells exposed to cytokines and 60% of the known T1D candidate genes are expressed in human islets.

The novelty of this study is the application, for the first time, of whole-genome RNA sequencing, to define all transcripts expressed in human pancreatic islets under basal conditions and following exposure to cytokines.

Whole-genome RNA sequencing is a new tool for transcriptomic studies, which does not require a priori knowledge of the targets. Combined with other tools, it also allows detection of splice variants. Sequencing was performed on RNA from human islets obtained from five organ donors, exposed or not to the proinflammatory cytokines IL-1 β and IFN- γ .

This approach allowed the identification of up to 15,200 genes expressed in the pancreas, thus doubling the number of previously known genes. Interestingly, 60% of the candidate genes for T1D were found to be expressed in the pancreas. Another interesting finding was that cytokines can modulate the expression of genes in the pancreas and this happens mainly by modifying alternative splicing in human islets. In addition, pancreatic cells themselves expressed cytokines and chemokines. Altogether these data suggest that there should be a close 'talk' between the immune system and pancreatic β -cells early in the natural history of T1D, leading to insulinitis and subsequent progression to diabetes.

The present study supports the value of whole-genome RNA sequencing for the identification of transcripts of specific cells/organs. In addition, this study provides a valuable dataset which could drive future genetic and functional studies in β -cells.

Reviews

The clinical potential of C-peptide replacement in type 1 diabetes

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Diabetes 2012;61:761–772

This review offers a comprehensive overview on what is known on C-peptide physiology and function.

It is well known that C-peptide fulfills an important function in the synthesis of insulin. However, there is now also extensive evidence highlighting that C-peptide is a hormone with its own effects and ability to bind to cell membranes and activate specific intracellular pathways.

Persistence of C-peptide has been associated with better metabolic control in newly diagnosed patients with T1D. In addition, residual C-peptide is also associated with a reduced risk of diabetic vascular complications. Several studies in animal models of diabetes and early clinical trials in patients with T1D have demonstrated that replacement of C-peptide results in beneficial effects on the diabetes-induced functional and structural abnormalities of peripheral nerves, kidneys, and brain. Based on this evidence, preservation or replacement of C-peptide is one of the key targets of new treatment strategies for improving outcomes in patients with diabetes. The development of long-acting C-peptide will facilitate further clinical trials and determine the potential role of C-peptide in the treatment of T1D.

Advanced glycation end products are direct modulators of β -cell function

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Diabetes 2011;60:2523–2532

Background: Advanced glycation end products (AGEs) are known to contribute to aging and chronic diseases. The aim of this study was to investigate whether AGEs also have a role in the development of T1D.

Methods: The effect of AGEs on insulin secretion was assessed in MIN6N8 cells and mouse islets and in vivo in three separate rodent models: AGE-injected or high AGE-fed Sprague-Dawley rats and non-obese diabetic (NODLt) mice. Rodents were also treated with the AGE-lowering agent alagebrium.

Results: β -Cell exposure to AGEs showed several abnormalities, including defects in acute glucose-stimulated insulin secretion, mitochondrial dysfunction characterized by increased superoxide generation, a decline in ATP content, loss of manganese superoxide dismutase (MnSOD) activity, reduced calcium flux, and increased glucose uptake. Interestingly, these abnormalities improved following treatment with alagebrium or with MnSOD adenoviral overexpression. Isolated mouse islets exposed to AGEs showed reduced glucose-stimulated insulin secretion, increased mitochondrial superoxide production, and depletion of ATP content. Again, all these abnormalities ameliorated following treatment with alagebrium or with the SOD mimetic, MnTBAP. In rats, transient or chronic exposure to AGEs caused progressive insulin secretory defects, superoxide generation, and β -cell death and these effects were improved by alagebrium. NODLt mice had increased circulating AGEs in association with an increase in islet mitochondrial superoxide generation, which was prevented by alagebrium, which also reduced the incidence of autoimmune diabetes. AGEs levels were also assessed in children, where they were found to be increased in those at risk of developing T1D compared to nonprogressors.

Conclusions: This study shows that AGEs can directly cause insulin secretory defects, most likely by impairing mitochondrial function, and in this way they may contribute to the development of T1D.

In last year's chapter, we commented on a study by Forbes et al., that reported that polymorphisms in the receptor for the AGEs (AGER) contributed to the susceptibility to T1D [14].

More recently the same group published this new study where the direct effect of AGEs on the development of T1D was investigated. Through a series of elegant in-vivo and in-vitro experiments, the authors convincingly showed that exposure to high levels of AGEs could significantly impair insulin secretion, and this effect was reversed by treatment with an AGEs antagonist. Interestingly, mitochondrial dysfunction, leading to increased oxidative stress, appeared to be a main mediator of AGEs effect on β -cells. However, further studies are required to understand whether oxidative stress is the sole mechanism mediating the negative effect of AGEs on β -cells or there are other mechanisms implicated.

Taken together, the results of the present study are in support of AGEs being implicated in the pathogenesis of T1D and thus being a potential target for new intervention strategies aiming at preserving β -cell function.

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