Type 1 Diabetes: Clinical and Experimental

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This 2013 chapter on type 1 diabetes (T1D) has the ambitious aim of summarizing the major breakthroughs in T1D basic and clinical research achieved during the last 12 months. Nineteen articles have been selected and commented on. As usual this selection has been difficult given the enormous amount of published papers, and the choice has been directed towards articles which in the authors' opinion are of interest because they highlight new aspects or confirm/expand previous findings related to T1D pathogenesis, prevention, treatment and complications.

The chapter starts with experimental studies summarizing major progress made in the understanding of the pathogenesis of T1D and highlighting the role of the gut microbiome and its interplay with sex hormones, the role of the innate immune system with a complex interplay between neutrophils, B-1a cells and plasmacytoid dendritic cells. Experimental studies have also allowed a better understanding of mechanisms implicated in podocytopathy in the context of diabetes, and in particular the role of mTOR pathways in the pathogenesis of nephropathy.

Major discoveries in the genetics field are also highlighted in this chapter and these include a genetic defect in SIRT1 associated with a monogenic form of T1D and a recent meta-analysis of genome-wide association studies on diabetic nephropathy.

The chapter also includes studies assessing new genetic, immunological and metabolic markers of disease onset and progression, as well as new data on fertility in people with T1D and new findings on the physiology of hypoglycemia.

Several studies aiming at improving the field of islet transplantation have been published during the last year and for this chapter we have just picked one key article highlighting the role of α_1 -antitrypsin in reducing inflammation and enhancing mouse pancreatic transplant survival.

With regard to T1D prevention and treatment, important results on the artificial pancreas system are reported together with data on the potential key role of the combined insulin pump-continuous glucose monitoring system in managing patients with poor glycemic control. In addition, negative results from two immunotherapy trials are discussed.

The chapter ends with a systematic review on one of the most feared complications of diabetes, diabetic ketoacidosis, showing a large between-country variation in its frequency at first presentation of T1D.

We do hope you will enjoy reading this chapter and find some useful information for your clinical practice or new ideas for your research.

Mechanism of the year

Gut microbiome protects male mice from T1D

Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity

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Science 2013;339:1084–1088

Background: Microbial exposures and sex hormones exert strong effects on autoimmune diseases, which are often more prevalent in women.

Methods and Results: Early-life microbial exposures influenced sex hormone levels and modified progression to autoimmunity in the non-obese diabetic (NOD) mouse model of T1D. Colonization by commensal microbes increased serum testosterone and protected NOD males from developing T1D. Transfer of

gut microbiota from adult males to immature females altered the recipient's microbiota and was associated with increased testosterone levels and metabolomic changes, decreased islet inflammation and autoantibody production, and protection from the development of T1D. These effects were strongly dependent on an intact androgen receptor activity.

Conclusions: Gut microbiome affects sex hormone levels and regulates T1D risk in mice with high genetic risk.

The gut microbiome is the central element assessed in the series of elegant and fascinating experiments performed by Markle and colleagues. Starting from the observation that autoimmunity presents a sex dimorphism with a higher incidence of some autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis, in females during the fertile age, the study proved that gut microbiome composition can explain gender differences in autoimmunity.

The experiments were performed in NOD mice which are genetically predisposed to develop T1D and in these mice there is a higher female frequency of diabetes. In the first part of the study it was shown that male NOD mice housed in specific pathogen-free (SPF) conditions were protected from T1D relative to females, whereas this was not the case in germ-free (GF) conditions, suggesting a role for the gut microbiome in the male protection from T1D.

Comparison of testosterone levels under SPF and GF conditions showed that levels in males were lower in GF than SPF conditions, whereas in females testosterone levels increased in GF conditions. These data suggested that commensal colonization regulates testosterone production and/or usage. Interestingly, the microbiome composition differences between sexes were evident only from the pubertal period onwards. Transfer of male microbiota, through gavage with cecal content, in female NOD weanlings altered gut microbiome and increased testosterone levels. These changes were prevented by treatment with flutamide, an androgen receptor antagonist. Further evidence for the role of the gut microbiome in modulating T1D risk emerged from the finding that the risk of developing T1D was significantly reduced in a cohort of female mice with modified microbiome followed longitudinally.

Overall, this study brilliantly shows that alterations in the gut microbiome composition in early life might suppress autoimmunity in animals at high risk for T1D, and highlights a strict bi-directionally interaction between the microbiome and sex hormones, in particular testosterone levels.

New mechanisms

The emerging role of innate immunity in T1D pathogenesis

Crosstalk between neutrophils, B-1a cells and plasmacytoid dendritic cells initiates autoimmune diabetes

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Nat Med 2013;19:65–73

Background: Type 1 diabetes is characterized by the destruction of insulin-producing pancreatic β -cells by autoreactive T cells. Up to now, the role of innate cells in the initiation of this disease remains poorly understood.

Methods and Results: In young female non-obese diabetic mice, physiological β -cell death induced the recruitment and activation of B-1a cells, neutrophils and plasmacytoid dendritic cells (pDCs) to the pancreas. Activated B-1a cells secreted IgGs specific for double-stranded DNA. IgGs activated neutrophils to release DNA-binding cathelicidin-related antimicrobial peptide (CRAMP), which bound self DNA. Self DNA, DNA-specific IgG and CRAMP peptide in turn activated pDCs through the Toll-like receptor 9-myeloid differentiation factor 88 pathway, leading to interferon- α production in pancreatic islets. Depletion of pDCs, or blocking the Toll-like receptor pathways responsible for triggering IFN- α production by pDCs, confirmed that B-1a cells, neutrophils and IFN- α -producing pDCs are required for the initiation of the T-cell response and T1D onset.

Conclusions: These findings demonstrate that an innate immune cell crosstalk occurs in the pancreas of young NOD mice and leads to the initiation of T1D.

T1D is well known to be a T-cell-mediated autoimmune disease, where β -cell damage is associated with the appearance of specific autoantibodies. This recent study by Diana et al. sheds more light on the pathogenesis of the disease and introduces new players in its context, represented by neutrophils, pDC and innate-like B-1a cells. Results from experiments in the NOD mice suggest that an interplay between these cells is among the earliest events in the pathogenesis of T1D. These cells appear to cooperate to induce IFN- α and other pro-inflammatory cytokines and chemokines in the islets of T1D-susceptible mice. The physiological waves of β -cell apoptosis occurring during the first weeks of postnatal life, both in mice and humans, could be the starting point for the activation of the innate immunity through the release of self DNA. This DNA forms immune complexes with specific IgG secreted by B-1a cells. Neutrophils in turn produce DNA-binding peptides, which potentiate the immune complexes and lead to the production of IFN- α by pancreatic pDCs.

The importance of these new intriguing findings is related to their potential translation into preventive strategies targeting these new 'actors' of the early phase of T1D pathogenesis. In order to reach this aim, one key point, as the authors point out, is to develop new tools able to allow the detection of these early events. In addition, it is important to understand whether a similar crosstalk between the same cells is involved in the onset of T1D in humans.

New mechanisms Understanding podocyte loss in diabetes

mTOR Regulates Nox4-Mediated Podocyte Depletion in Diabetic Renal Injury

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Diabetes 2013 (E-pub ahead of print)

Background: Podocyte apoptosis is a key mechanism implicated in excessive loss of urinary albumin. Pharmacological doses of the mTOR inhibitor rapamycin reduce albuminuria in diabetes. The aim of this study was to test the hypothesis as to whether mTOR mediates podocyte damage in diabetes.

Methods and Results: High glucose (HG) induced apoptosis of podocytes, inhibited AMPK activation, inactivated tuberlin and activated mTOR. HG also increased the levels of Nox4 and Nox1 and NADPH oxidase activity. Inhibition of mTOR by low-dose rapamycin decreases HG-induced Nox4 and Nox1, NADPH oxidase activity and podocyte apoptosis. Inhibition of mTOR had no effect on AMPK or tuberlin phosphorylation indicating that mTOR is downstream of these signaling molecules. A similar decrease in the activation of AMPK and tuberlin and activation of mTOR with increase in Nox4 and NADPH oxidase activity was detected in isolated glomeruli of OVE26 mice. mTOR inhibition by small dose of rapamycin reduced podocyte apoptosis, attenuated glomerular injury and albuminuria.

Conclusions: These results support a novel function of mTOR in Nox4-derived ROS generation and podocyte apoptosis that contributes to urinary albumin excretion in T1D. This suggests that mTOR and/or NADPH oxidase inhibition may represent new treatment strategies for diabetic kidney disease.

The pathogenesis of albuminuria in people with T1D is complex and derives from the interplay of several metabolic and hemodynamic factors acting on glomerular components. Podocytes are key elements of the glomerular barrier and their apoptosis seems to be one of the key events leading to glomerular injury and albuminuria [1].

Using mouse podocytes and a T1D mice model, Eid et al. were able to show that high glucose levels can induce podocyte apoptosis through mTOR-dependent mechanisms. mTOR activation was associated with increased oxidative stress through the upregulation of Nox4 and Nox1 expression and

NADPH oxidase activity. The role of mTOR in inducing podocyte injury was confirmed by the subsequent finding that the mTOR inhibitor rapamycin reduced podocyte injury and albuminuria. Overall these findings reveal additional mechanisms implicated in podocyte injury in the context of diabetes and suggest new potential therapies directed at protecting the podocyte and reducing albuminuria, represented by direct mTOR inhibitors or antioxidants targeting Nox4.

New paradigms Fecal microbiota in children with β -cell autoimmunity

Fecal microbiota composition differs between children with β -cell autoimmunity and those without

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Diabetes 2013;62:1238–1244

Background: The role of the intestinal microbiota as a regulator of autoimmune diabetes in animal models is well established, but data on human T1D are scant.

Methods: The intestinal microbiota composition was assessed using pyrosequencing in 18 children with at least two diabetes-associated autoantibodies and in 18 autoantibody-negative controls matched for age, sex, early feeding history, and HLA risk genotype.

Results: Principal component analysis indicated that a low abundance of lactate-producing and butyrate-producing species was associated with β -cell autoimmunity. The two most dominant *Bifidobacterium* species, *Bifidobacterium adolescentis* and *Bifidobacterium pseudocatenulatum*, were decreased, whereas there was an increased abundance of the *Bacteroides* genus in stools of children with autoantibodies. No increase in fecal calprotectin or IgA as marker of inflammation was found in children with autoantibodies.

Conclusions: These data emphasize the importance of bifidobacteria and lactate- or butyrate-producing species in relation to the development of β -cell autoimmunity. Functional studies are required to further investigate the role of the low abundance of bifidobacteria and butyrate-producing species in adversely affecting the intestinal epithelial barrier function and inflammation, and to understand the role of the *Bacteroides* genus in development of T1D.

During recent years, an increasing number of studies have suggested a potential role of the gut microbiota in the pathogenesis of T1D [2]. The gut microbiota is a complex environment which has been strongly linked to the immune system, being involved in the regulation of immune responses. The recent study by de Goffau et al. reports data emerged from the comparison of 18 children with two or more diabetes specific autoantibodies and 18 controls with similar HLA risk genotype for T1D, in order to exclude its potential confounding effect on the gut composition.

Overall the main finding of this study is that children with multiple autoantibodies have decreased levels of bacteria producing lactate and butyrate, particularly in the late phases of prediabetes, as indicated by lower levels particularly in children with three or four autoantibodies. This finding is of particular importance in the light of previous data showing that butyrate might be beneficial for colonic epithelial cells and it might regulate the assembly of tight junctions and thus gut permeability [3, 4]. Interestingly, increased gut permeability has been found to precede the development of T1D [5].

In contrast, there were no significant differences in markers of intestinal inflammation, such as fecal calprotectin and IgA, suggesting that intestinal inflammation, at least in this study population, is not a key feature of the prediabetes status.

Although this study is based on a small sample size, it definitely reaches important results, which need to be further explored in larger studies including longitudinal collections of stool samples in order to have a clearer picture of the over-time changes in gut microbiota, particularly those occur-

ring before the onset of T1D. This is of particular importance for a better definition of the disease pathogenesis and also as a starting point to develop preventive trials with agents acting at the gut microbiota composition level.

New paradigms

Hyperglycemia induces brain zone-specific microstructural changes

White matter microstructural integrity in youth with type 1 diabetes

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Diabetes 2013;62:581–589

Background: In youth with T1D there is evidence of decreased white and gray matter volumes, but the effect of hyperglycemia on white matter integrity has not been quantitatively assessed.

Methods: Diffusion tensor imaging (DTI), using two complimentary approaches (region-of-interest and voxelwise tract-based spatial statistics) to quantify white matter integrity, was performed in a retrospective study of youth with T1D ($n = 73$) and control participants ($n = 30$). Exposure to chronic hyperglycemia, severe hyperglycemic episodes, and severe hypoglycemia were estimated through medical records review, HbA_{1c} levels, and interviews of parents and youth.

Results: Fractional anisotropy (which indicates the directionality of water molecule movement) was lower in the superior parietal lobule, whereas mean diffusivity (which indicates the average magnitude of water molecule movement in the three axes) was reduced in the thalamus of youth with T1D. A history of ≥ 3 severe hyperglycemic episodes was associated with reduced anisotropy and increased diffusivity in the superior parietal lobule and increased diffusivity in the hippocampus.

Conclusions: These findings indicate that microstructural integrity of white matter are additional brain alterations present in youth with T1D, and suggest vulnerability of the superior parietal lobule, hippocampus, and thalamus to glycemic extremes during brain development.

The effect of diabetes on the developing brain is a topic of particular interest, which however is not fully understood and is complicated by some controversial results, emerged from different studies [6, 7]. Recent data indicate subtle decrements in the overall IQ of children with T1D, particularly in those with an early onset of the disease [8]. Therefore, identifying subtle changes in the central nervous system (CNS) gray and/or white matter and their relationship with high or low glucose levels or with glucose fluctuations would be of invaluable help to prevent cognitive problems in youth with T1D.

Advances in the field of magnetic resonance imaging offer the opportunity of studying in detail the gray and white matter and identifying early subtle abnormalities.

In the present study the authors used DTI, a technique based on the measurement of water molecule movement in the brain, to assess the CNS and identify changes associated with hypo- or hyperglycemia. The main study findings were that adolescents with T1D had more evident white matter changes in the superior parietal lobules and hippocampus when compared to controls, in line with previous studies showing reduced white and gray matter in this region associated with hyperglycemia [7]. In the present study, DTI measures were particularly impaired in subjects with three or more episodes of hyperglycemia, further supporting the effect of high glucose levels probably through an activation of mitochondrial oxidative pathways. Another key observation was reduced diffusivity in the thalamus associated with hyperglycemia. In contrast, there were no specific key findings related to hypoglycemia. These latter findings appear different from the results of previous studies based on volumetric assessments. Therefore, this highlights that different techniques can help in detecting different aspects related to abnormal glycemic control.

The study presents the limitation of a potential bias related to the retrospective way of collecting data on episodes of hyper- or hypoglycemia. Nevertheless, it opens the way to ideas for future prospective studies, which should aim to apply neuroimaging assessments in children since T1D diagnosis and correlate them with data on glycemic control collected in an unbiased way.

IL-7 receptor blockade reverses autoimmune diabetes by promoting inhibition of effector/memory T cells

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Proc Natl Acad Sci USA 2012;109:12668–12673

Background: To protect the organism against autoimmunity, self-reactive effector/memory T cells ($T_{E/M}$) are controlled by cell-intrinsic and -extrinsic regulatory mechanisms. However, how some $T_{E/M}$ cells escape regulation and cause autoimmune disease is currently not understood.

Methods and Results: Blocking IL-7 receptor- α (IL-7R α) with monoclonal antibodies in non-obese diabetic (NOD) mice prevented autoimmune diabetes and reversed disease in new-onset diabetic mice. IL-7-deprived diabetogenic $T_{E/M}$ cells were still present in treated animals but showed increased expression of the inhibitory receptor Programmed Death 1 (PD-1) and reduced IFN- γ production. In contrast, IL-7 suppressed PD-1 expression on activated T cells in vitro. Adoptive transfer experiments revealed that $T_{E/M}$ cells from anti-IL-7R α -treated mice had lost their pathogenic potential, indicating that absence of IL-7 signals induces cell-intrinsic tolerance. In addition to this mechanism, IL-7R α blockade altered the balance of regulatory T cells and $T_{E/M}$ cells, thus promoting cell-extrinsic regulation and further increasing the threshold for diabetogenic T-cell activation.

Conclusions: These findings support a role for IL-7 in the pathogenesis of autoimmune diabetes and suggest IL-7R α blockade as a therapy for established T-cell-dependent autoimmune diseases.

Anti-IL-7 receptor- α reverses established type 1 diabetes in non-obese diabetic mice by modulating effector T-cell function

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Proc Natl Acad Sci USA 2012;109:12674–12679

Background: Genetic variation in the IL-7R α gene is associated with susceptibility to T1D. The aim of this study was to investigate the therapeutic efficacy and mechanism of IL-7R α antibody in a mouse model of T1D.

Methods and Results: In newly onset diabetic mice IL-7R α antibody induced complete remission after only two to three injections. IL-7 increase, whereas IL-7R α antibody therapy reduced the number of CD4⁺ (T_H1) and CD8⁺ cells producing IFN- γ . In contrast, IL-7 decreased and IL-7R α antibody increased the inhibitory receptor PD-1 expression in the effector T cells. PD-1 blockade reversed the immune tolerance induced by the IL-7R α antibody. IL-7R α antibody therapy increased the frequency of regulatory T cells without affecting their suppressor activity.

Conclusions: These data showed that IL-7R α antibody treatment can revert newly onset T1D and prevent its development. Therefore, IL-7R α antibody treatment might be a unique disease-modifying approach to T1D.

IL-7 is a key cytokine with an important role in T-cell homeostasis, which may contribute to T1D pathogenesis, by reverting suppressor functions in T cells [9]. The studies by Lee et al. and Penaranda et al. independently reached a similar conclusion: in mice models blockade of the IL-7 signaling can revert recent-onset diabetes and prevent diabetes development.

Blockade of IL-7 receptor was associated with increased expression of the inhibitory PD-1 protein on T cells and blockade of the PD-1 pathway abrogates the protective effect of IL-7R α inhibition. Therefore, it emerges that IL-7 and PD-1 act as antagonists in the regulation of T-cell responses. This is in line with previous data indicating that loss of PD-1 is associated with increased levels of IFN- γ production and progression to T1D [10].

Overall these results propose an interesting new link among IL-7, PD-1, and autoimmunity and lead the way to a new potential target for preventive strategies, with drugs specifically targeting IL-7R, alone or in combination with other targets.

New concerns

Reduced number of live births in people with T1D

Fertility in people with childhood-onset type 1 diabetes

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Diabetologia 2013;56:78–81

Background: To assess the number of live births in a population-based, retrospective cohort of women and men with childhood-onset T1D, and matched controls.

Methods: Information on the reproductive histories of a Finnish cohort of 2,307 women and 2,819 men with T1D and matched controls was obtained from the National Population Register data. Cases with diabetes were diagnosed between 1965 and 1979 at the age of 17 or under.

Results: Both women and men with diabetes had fewer live births than controls; the hazard ratio of having a first child for diabetic women compared with controls was 0.66 (95% CI 0.62–0.71) and for men was 0.77 (95% CI 0.72–0.83). The difference between diabetic women and controls as regards having children was significantly smaller in more recent than in earlier cohorts. Later age at onset of diabetes was associated with a higher rate of having a first child among men ($p = 0.04$) and having a second live birth among women ($p = 0.002$).

Conclusions: T1D affects the number of live births in both women and men. The age at onset of diabetes is associated with the pattern of reproduction in both diabetic women and men.

In this retrospective study the authors assessed fertility in a cohort of Finnish women and men with a diagnosis of T1D when aged 17 years or less during the period 1965–1979 and found a significantly lower number of live births when compared to matched controls. This difference was significant for the first child and, in women, was even higher for a second child. Potential factors explaining the lower fertility in people with T1D might be menstrual problems associated with T1D, such as menstrual irregularities, long cycles [11]; whereas in men fertility might be affected by a higher prevalence of erectile dysfunction and lower quality of semen in those with diabetes [12].

In the past, the fear of passing diabetes to the offspring or the fear of the risk associated with diabetic pregnancies might have influenced the choice of women or men with T1D of not having children, and therefore contributed to the reported lower fertility. This is partly supported by the study finding that the differences in fertility between people with T1D and controls in the Finnish cohort have decreased over more recent years, probably reflecting that the misconception of diabetes being an obstacle to pregnancy has disappeared over time.

Early loss of the glucagon response to hypoglycemia in adolescents with type 1 diabetes

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Diabetes Care 2012;35:1757–1762

Background: To assess the glucagon response to hypoglycemia and identify influencing factors in patients with T1D compared with control subjects.

Methods: Hyperinsulinemic hypoglycemic clamp studies were performed in all participants. Glucagon response to both hypoglycemia and arginine was measured, along with epinephrine, cortisol, and growth hormone responses to hypoglycemia. Fasting and stimulated C-peptide were used to assess residual β -cell.

Results: The study population comprised 28 non-obese adolescents with T1D (14 female, mean age 14.9 years (range 11.2–19.8), median T1D duration: 0.66 years (range 0.01–9.9)) and 12 healthy control subjects (6 female, 15.3 years (12.8–18.7)). The glucagon peak to arginine stimulation was similar between the two groups ($p = 0.27$). In contrast, the glucagon peak to hypoglycemia was lower in T1D adolescents (95% CI): 68 (62–74) vs. 96 (87–115) pg/ml ($p < 0.001$). This response was >3 SDs from baseline for only 7% of subjects with T1D compared with 83% of control subjects and was lost at a median duration of diabetes of 8 months and as early as 1 month after diagnosis. No association was found between the glucagon response and height, weight, BMI, or HbA_{1c}. Epinephrine, cortisol, and growth hormone responses to hypoglycemia were present in both groups.

Conclusions: The glucagon response to hypoglycemia in adolescents with T1D is influenced by the duration of diabetes and can be lost early in the course of the disease.

Hypoglycemia is a frequent complication among children and adolescents with T1D and a common fear among their parents, which influences diabetes management.

A blunted counter-regulatory response has been reported in youth with T1D, and this includes loss of glucagon response after a variable T1D duration. The novelty of this recent physiological study is in reporting an impaired glucagon response early during the course of diabetes. Duration of diabetes emerged to significantly influence the loss of glucagon response to hypoglycemia, which occurred at a median duration of 8 months after diabetes onset, and in some study participants even as early as 1 month after diagnosis. This latter finding is alarming and indicates that the risk of hypoglycemia with a blunted counter-regulatory response should be considered soon after T1D diagnosis.

As rightly pointed out by the authors, there is now a need for prospective studies in children and adolescents starting from the onset of diabetes, to further characterize the mechanisms influencing the over-time change in the glucagon response to hypoglycemia and identify whether there is a way of preventing this loss.

Characteristics of rapid vs. slow progression to type 1 diabetes in multiple islet autoantibody-positive children

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Diabetologia 2013;56:1615–1622

Background: Islet autoantibody-positive children progress to T1D at variable rates. The aim of this study was to assess whether characteristic autoantibody and/or gene profiles could be defined for phenotypes showing extreme progression.

Methods: Autoantibodies to insulin (IAA), GAD (GADA), insulinoma-associated antigen-2 (IA-2A) and zinc transporter 8 (ZnT8A) were measured in follow-up sera, and genotyping for T1D susceptibility genes (HLA-DR/HLA-DQ, INS VNTR, PTPN22, PTPN2, ERBB3, IL2, SH2B3, CTLA4, IFIH1, KIAA0350, CD25, IL18RAP, IL10, COBL) was performed in children born to a parent with T1D and prospectively followed from birth for up to 22 years.

Results: Of the 1,650 children followed, 23 developed multiple autoantibodies and progressed to diabetes within 3 years (rapid progressors), whereas 24 children developed multiple autoantibodies and remained non-diabetic for more than 10 years from seroconversion (slow progressors). No differences between the two groups were found in HLA-DR/HLA-DQ genotypes and development of IAA, GADA and ZnT8A. In contrast, IA-2A development was significantly delayed in the slow progressors. In addition, the combined presence or absence of T1D susceptibility alleles of non-HLA genes, particularly IL2, CD25, INS VNTR, IL18RAP, IL10, IFIH1 and PTPN22, allowed the separation between the two groups, and discrimination was improved among children carrying high-risk HLA-DR/HLA-DQ genotypes.

Conclusions: These data suggest that genotypes of non-HLA T1D susceptibility genes influence the likelihood or rate of diabetes progression among children with multiple islet autoantibodies.

One important issue in the application of preventive strategies for complex diseases, such as T1D, is the identification of subjects at particular risk, who could benefit from the implementation of more aggressive therapeutic strategies.

The availability of a large cohort of 'at-risk' subjects for the development of T1D, represented by subjects with a first-degree relative with T1D followed in the BABYDIAB study, with a long period of follow-up to 20 years, allowed the identification of two groups of extreme phenotypes in terms of progression among subjects with multiple autoantibodies. When comparing subjects with multiple autoantibodies who rapidly progressed to T1D with those who remained disease-free at 10-year follow-up, it clearly emerged that discriminators between the two groups were IA-2A autoantibodies and non-HLA genotypes. In particular, IA-2A autoantibodies, which generally indicate a more aggressive disease and appear more closely to T1D onset, manifested earlier in the rapid progressors. Even more interesting was the finding that the two groups could be distinguished by a combination of presence/absence of non-HLA genotypes of IL2, CD25, IL10, IFIH1, INS VNTR, IL18RAP and PTPN22. Although the study results are based on a small sample size and on the assessment of only a subset of potential T1D susceptibility genes, the study findings are relevant given that they highlight that there is a potential of distinguishing rapid versus slow progressors to T1D among highly predisposed individuals. This represents an important starting point for the application of preventive tailored strategies.

Meta-immunological profiling of children with type 1 diabetes identifies new biomarkers to monitor disease progression

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Diabetes 2013;62:2481–2491

Background: Triggers of islet autoimmunity, time course and the precise mechanisms responsible for the progressive β -cell failure in T1D are not completely understood. Understanding the link between metabolism and immune tolerance could lead to the identification of new markers for the monitoring of disease onset and progression.

Methods: Several immune cell subsets and factors with high metabolic impact as markers associated with disease progression were studied in high-risk subjects, T1D patients at onset, 12 and 24 months after diagnosis. A multiple correlation matrix among different parameters was evaluated statistically and assessed visually on two-dimensional graphs. Markers to predict residual β -cell function up to 1 year after diagnosis were identified in multivariate logistic regression models.

Results: The meta-immunological profile significantly changed over time in patients with T1D and a specific signature associated with disease worsening was identified. A multivariate logistic regression model including age, BMI, fasting C-peptide, number of circulating $CD3^+CD16^+CD56^+$ cells and the percentage of $CD1c^+CD19^-CD14^-CD303^-$ type 1 myeloid dendritic cells (mDC1s) at disease onset had a significant predictive value.

Conclusions: The identification of a specific meta-immunological profile associated with disease status may contribute to understand the basis of diabetes progression.

The study aimed to identify metabolic and immunological parameters for the prediction of residual β -cell function over time, by comparing three groups of subjects: 29 children at risk of T1D diabetes, 114 children with new onset T1D and 34 controls. The meta-immunological profiling revealed 3 circulating immune cell populations and 4 metabolic/inflammatory markers being significantly different among the three study groups. $CD8^+$ T cells were higher in 'at-risk' subjects compared to those with T1D or controls, supporting previous findings of proliferation of these cells during the development of insulinitis. In contrast, memory cells were higher in children with T1D at disease onset, and their number declined over time, probably reflecting gradual exhaustion of islet-specific T-cell clones. Plasmacytoid dendritic cells were also higher in children with diabetes and this reinforces their role in presenting autoantigens and maintaining the immune response.

With regard to metabolic parameters, children with T1D showed decreased leptin levels and increased levels of its soluble receptor (sLepR). Levels of sLepR declined over time and were inversely related to C-peptide levels, leading the authors to hypothesize that sLepR might represent an early novel marker of T1D. Of particular interest was the finding that two specific immune cell populations ($CD3^+CD16^+CD56^+$ and mDC1s) at disease onset were able to increase the prediction of β -cell function decline, as assessed by C-peptide levels at 12 and 24 months after disease onset, when included in a predictive model with other known parameters such as age, BMI and fasting C-peptide at diagnosis.

The use and efficacy of continuous glucose monitoring in type 1 diabetes treated with insulin pump therapy: a randomized controlled trial

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Diabetologia 2012;55:3155–3162

Background: The aim of this multicenter, randomized, controlled crossover study was to determine the efficacy of adding continuous glucose monitoring (CGM) to insulin pump therapy (CSII) in patients with T1D.

Methods: Children and adults ($n = 153$) on CSII with HbA_{1c} 7.5–9.5% (58.5–80.3 mmol/mol) were randomized to a sensor-on or sensor-off arm for 6 months. After 4 months' washout, participants crossed over to the other arm for 6 months. The primary outcome was the difference in HbA_{1c} levels between arms after 6 months.

Results: 77 participants were randomized to the on/off sequence and 76 to the off/on sequence. The mean difference in HbA_{1c} was -0.43% (-4.74 mmol/mol) in favor of the sensor-on arm (8.04% (64.34 mmol/mol) vs. 8.47% (69.08 mmol/mol); 95% CI -0.32% , -0.55% (-3.50 , -6.01 mmol/mol); $p < 0.001$). Following cessation of glucose sensing, HbA_{1c} reverted to baseline levels. Time spent with sensor glucose < 3.9 mmol/l was shorter during the sensor-on arm than in the sensor-off arm (19 vs. 31 min/day; $p = 0.009$). The number of daily boluses increased in the sensor-on arm (6.8 ± 2.5 vs. 5.8 ± 1.9 , $p < 0.0001$), along with the frequency of use of the temporary basal rate (0.75 ± 1.11 vs. 0.26 ± 0.47 , $p < 0.0001$) and manual insulin suspend (0.91 ± 1.25 vs. 0.70 ± 0.75 , $p < 0.018$) functions. There was no difference in the occurrence of severe hypoglycemia episodes between the sensor-on and sensor-off arms (4 vs. 2, $p = 0.40$).

Conclusions: CGM decreased HbA_{1c} levels and time spent in hypoglycemia in individuals with T1D using CSII. More frequent self-adjustments of insulin therapy may have contributed to these effects.

Improving glycemic control is a central issue in the management of patients with T1D. Sensor augmented insulin pump therapy has been introduced in the last years in an attempt to improve glycemic control. However, there are conflicting results on the effect on glycemic control associated with the implementation of this new system in patients on CSII. In this context, the present study offers good evidence for a significant improvement in glycemic control, as assessed by HbA_{1c} , by the addition of a CGM in children and adults with T1D and baseline poor glycemic control on insulin pump therapy. The improvement in HbA_{1c} was also associated with increased time spent in normoglycemia. These improvements were likely the consequence of more frequent self-adjustments of insulin therapy in the sensory augmented insulin pump arm, as documented by more frequent insulin bolus administration together with more frequent use of temporary basal rates and the manual basal suspend function.

Clinical trials, new treatments

The artificial pancreas wins in a diabetes camp

Nocturnal glucose control with an artificial pancreas at a diabetes camp

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N Engl J Med 2013;368:824–833

Background: Recent studies have found that an artificial pancreas system can improve glucose control and reduce nocturnal hypoglycemia. However, it is not known whether such results can be replicated in settings outside the hospital.

Methods: In a multicenter, multinational, randomized, crossover trial, the short-term safety and efficacy of an artificial pancreas system for control of nocturnal glucose levels in patients (10–18 years of age) with T1D at a diabetes camp was assessed. 56 patients were randomized to receive treatment with an artificial pancreas on a first night and a sensor-augmented insulin pump (control) on a second night or to the reverse order of therapies on the first and second nights. The primary endpoints were the number of hypoglycemic events (sensor glucose <63 mg/dl (3.5 mmol/l) for at least 10 consecutive min), the time spent with glucose levels <60 mg/dl (3.3 mmol/l), and the mean overnight glucose level for each patient.

Results: On artificial pancreas nights, there were significantly fewer episodes of glucose levels <63 mg/dl (7 vs. 22) and significantly shorter periods when glucose levels were <60 mg/dl ($p = 0.003$ and $p = 0.02$, respectively, after adjustment for multiplicity). Median values for the individual mean overnight glucose levels were 126.4 mg/dl (interquartile range (IQR) 115.7–139.1 (7.0 mmol/l; IQR 6.4–7.7)) with the artificial pancreas and 140.4 mg/dl (IQR 105.7–167.4 (7.8 mmol/l; IQR 5.9–9.3)) with the sensor-augmented pump. No serious adverse events were reported.

Conclusions: Treating patients at a diabetes camp with an artificial pancreas system reduced nocturnal hypoglycemia and improved glucose control when compared with a sensor-augmented insulin pump.

Over the last years, several studies have contributed to the conclusion that artificial pancreas systems represent a valuable means to control nocturnal glucose levels both in adults and children with T1D [13]. This is important given that nocturnal hypoglycemia is a common complication in youth with T1D, in whom high rates of severe episodes occur at night, and are often undetected (hypoglycemia unawareness).

There is clear evidence that within ‘hospital settings’ the artificial pancreas systems can reduce the time spent with low glucose levels, however a big challenge is to show similar findings in the ‘home setting’, reflecting daily life of people with T1D.

The study by Phillip and colleagues represents a further step towards the implementation of the artificial pancreas, with a study setting in-between the hospital and the home settings: a diabetic camp. Diabetes camps are close to real life in terms of activities but there is still the ‘supervision’ of a healthcare team. The study compared the MD-Logic artificial pancreas — a closed-loop system with real-time alarms — with a sensor-augmented pump system in 56 children with T1D, aged between 10 and 18 years across three diabetes camps in Israel, Slovenia and Germany over 3 days.

Although the results are based on a small number of subjects and on an assessment performed over a short period of time, they are of utmost importance as they confirm that previous findings of reduced overnight hypoglycemia within a hospital setting might eventually be translated to the home setting.

Clinical trial

New preventive trials, but further negative results!

Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicenter, randomized, double-blind, placebo-controlled trials

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Lancet 2013;381:1905–1915

Background: Innate immunity contributes to the pathogenesis of T1D, however there are no randomized controlled trials assessing the effect of blockade of the key innate immune mediator interleukin-1 (IL-1). The authors report two trials using canakinumab, a human monoclonal anti-IL-1 antibody, or anakinra, a human IL-1 receptor antagonist, to improve β -cell function in recent-onset T1D.

Methods: Two randomized, placebo-controlled trials in two groups of patients with recent-onset T1D and mixed-meal tolerance-test-stimulated C-peptide of at least 0.2 nM were performed. In the canakinumab trial, patients aged 6–45 years, enrolled at 12 sites in the USA and Canada, were randomized to subcutaneous injection of either 2 mg/kg (max. 300 mg) canakinumab or placebo monthly for 12 months. In the anakinra trial, patients aged 18–35 years, enrolled at 14 sites across Europe, were randomized to 100 mg anakinra or placebo daily for 9 months.

The primary endpoint was baseline-adjusted 2 h area under curve C-peptide response to the mixed meal tolerance test at 12 months (canakinumab trial) and 9 months (anakinra trial). Analyses were by intention to treat.

Results: 69 patients were randomly assigned to canakinumab ($n = 47$) or placebo ($n = 22$) monthly for 12 months, and 69 were randomly assigned to anakinra ($n = 35$) or placebo ($n = 34$) daily for 9 months. 45 canakinumab-treated and 21 placebo-treated patients in the canakinumab trial and 25 anakinra-treated and 26 placebo-treated patients in the anakinra trial were included in the primary analyses. There was no significant difference in C-peptide area under curve between the canakinumab and placebo groups at 12 months (0.01 nmol/l (95% CI -0.11 to 0.14 ; $p = 0.86$)), nor between the anakinra and the placebo groups at 9 months (0.02 nmol/l (-0.09 to 0.15 ; $p = 0.71$)). The number and severity of adverse events did not differ between groups in the canakinumab trial. In the anakinra trial, patients in the anakinra group had significantly higher grades of adverse events than the placebo group ($p = 0.018$), represented mainly by injection site reactions.

Conclusions: Canakinumab and anakinra were not effective as single immunomodulatory drugs in recent-onset T1D, although they showed safe profiles.

The observation that substantial β -cell function still persists at the time of T1D onset has prompted efforts to preserve β -cells in newly diagnosed patients. The majority of previous trials have focused on the adaptative immune system, by testing molecules targeting T or B lymphocytes, such as anti-CD3, abatacept, anti-CD20. Overall, those treatments were associated with a transient stabilization of β -cell function for only 6–12 months.

Recently, there has been increasing interest in the role of the innate immune system in the pathogenesis of T1D (see paper by Diana et al. above). Special attention has focused on IL-1 β , a pro-inflammatory molecule, with a β -cell proapoptotic effect and mediating β -cell glucotoxicity. In addition, IL-1 β can amplify the adaptative immune responses [14].

These observations and the results of a preliminary trial led the way to the two recent trials with canakinumab and anakinra. The results of these trials were disappointing, with lack of effects on β -cell function, glycemic control and insulin requirements, although the safety profiles of both drugs were acceptable.

The lack of success of these new trials follows the same trend of previous trials of immunomodulatory drugs. However, as the authors point out, the study conclusions should not refrain from designing further trials with these drugs. One potential limitation was the inclusion of patients after the onset of T1D. Based on the observation that levels of IL-1 β are high at diagnosis and even before, the application of IL-1 β inhibitors could be useful mainly during the prediabetes phase or should at least be started at the time of T1D diagnosis.

Another important point is that the use of a single drug is probably not sufficient in diseases with a complex pathogenesis. Strategies to preserve β -cell function based on a combination of drugs may be more effective, but could increase the likelihood of adverse effects. Therefore, these two recent trials should not dampen the enthusiasm around a potential effect of the inhibition of IL-1 β , but they should be further assessed at earlier stages (prediabetes) and/or in combination with other immunomodulatory drugs.

Identification of a SIRT1 mutation in a family with type 1 diabetes

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Cell Metab 2013;17:448–455

Background: T1D is caused by autoimmune-mediated β -cell destruction leading to insulin deficiency. The histone deacetylase SIRT1 plays an essential role in modulating several age-related diseases.

Methods and Results: The authors report a family carrying a mutation in the SIRT1 gene, in which 5 affected members developed an autoimmune disorder: 4 developed T1D and 1 ulcerative colitis. The index case was a 26-year-old man, presenting with the typical features of T1D (lean body mass, autoantibodies, T-cell reactivity to β -cell antigens, and a rapid dependence on insulin). Direct and exome sequencing identified a T-to-C exchange in exon 1 of SIRT1, corresponding to a leucine-to-proline amino acid change at residue 107. Expression of SIRT1-L107P in insulin-producing cells resulted in overproduction of nitric oxide, cytokines, and chemokines.

Conclusions: These data support a role for SIRT1 in human autoimmunity and suggest a monogenic form of T1D associated with mutations in this gene.

T1D is a multifactorial disease with a polygenic component. Up to now the only cases of T1D due to a single gene mutation have been reported in the context of complex phenotypes due to mutations in the AIRE and FOXP3 genes.

The study by Biason-Lauber et al. reveals a new monogenic form of T1D due to a mutation in the SIRT1 gene. The discovery was made after studying an intriguing family where the index case presented with features of T1D combined with insulin resistance. Similar characteristics were noticed in other 3 family members. A fifth family member presented another autoimmune disease, ulcerative colitis. The family history suggested a pattern of autosomal dominant inheritance.

To gain more understanding on the potential role of SIRT1 in the pathogenesis of T1D, experimental studies on cell cultures and animal models were performed, which showed that SIRT1 can modulate β -cell function. When the mutant SIRT1 was expressed in β -cells, it led to an increased expression of nitric oxide, TNF- α and the chemokine KC, which exerted negative effects on the β -cells. In these mice models, removal of SIRT1 was associated with higher rates of β -cell death and increased risk of developing T1D. In addition, expression of the SIRT1 mutant in myoblasts induced insulin resistance.

Previous animal studies had suggested a role of SIRT1 in regulating metabolism and in protecting against age-related diseases. In addition, there are also specific data on the expression of this gene in the β -cells, and on its involvement in the regulation of insulin secretion as well as insulin sensitivity [15, 16].

Overall these findings point towards a potential new T1D gene, which seems to be directly implicated in the regulation of β -cell function, along with an effect on insulin sensitivity. Although it might be too early to consider SIRT1 as a definitive T1D gene, these results could eventually lead to a better understanding of the pathogenesis of T1D and new therapeutic targets.

New susceptibility loci associated with kidney disease in type 1 diabetes

Sandholm N, Salem RM, McKnight AJ, Brennan EP, Forsblom C, Isakova T, McKay GJ, Williams WW, Sadlier DM, Makinen VP, Swan EJ, Palmer C, Boright AP, Ahlqvist E, Deshmukh HA, Keller BJ, Huang H, Ahola AJ, Fagerholm E, Gordin D, Harjutsalo V, He B, Heikkila O, Hietala K, Kyto J, Lahermo P, Lehto M, Lithovius R, Osterholm AM, Parkkonen M, Pitkanieni J, Rosengard-Barlund M, Saraheimo M, Sarti C, Soderlund J, Soro-Paavonen A, Syreeni A, Thorn LM, Tikkanen H, Tolonen N, Tryggvason K, Tuomilehto J, Waden J, Gill GV, Prior S, Guiducci C, Mirel DB, Taylor A, Hosseini SM, Parving HH, Rossing P, Tarnow L, Ladenvall C, Alhenc-Gelas F, Lefebvre P, Rigalleau V, Roussel R, Tregouet DA, Maestroni A, Maestroni S, Falhammar H, Gu T, Mollsten A, Cimponeriu D, Ioana M, Mota M, Mota E, Serafinceanu C, Stavarachi M, Hanson RL, Nelson RG, Kretzler M, Colhoun HM, Panduru NM, Gu HF, Brismar K, Zerbini G, Hadjadj S, Marre M, Groop L, Lajer M, Bull SB, Waggott D, Paterson AD, Savage DA, Bain SC, Martin F, Hirschhorn JN, Godson C, Florez JC, Groop PH, Maxwell AP

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PLoS Genet 2012;8:e1002921

Background: Diabetic nephropathy (DN) is a major complication of diabetes and the leading cause of end-stage renal disease (ESRD). There is evidence suggesting a genetic component in the pathogenesis of DN. However, the genes and the molecular mechanisms remain poorly understood.

Methods: A two-stage study design was applied, in which a genome-wide association study (GWAS) was performed using a set of three discovery cohorts in the Genetics of Nephropathy: an International Effort (GENIE) consortium, and the top signals for DN and ESRD were analyzed further in the second phase set of 9 independent cohorts with 5,873 patients in total.

Results: Combined meta-analysis identified two SNPs associated with ESRD: rs7583877 in the *AFF3* gene ($p = 1.2 \times 10^{-8}$) and an intergenic SNP on chromosome 15q26 between *RGMA* and *MCTP2*, rs12437854 ($p = 2.0 \times 10^{-9}$). Functional data suggested that *AFF3* influences renal tubule fibrosis via the transforming growth factor- β (TGF- β_1) pathway. The strongest association with DN was seen for an intronic SNP in the *ERBB4* gene (rs7588550, $p = 2.1 \times 10^{-7}$), a gene with type 2 diabetes DN differential expression and in the same intron as a variant associated with expression of *ERBB4*.

Conclusions: All these detected associations represent new signals in the pathogenesis of DN.

Kidney disease, a well-known complication of T1D, is a key determinant of morbidity and mortality in patients with diabetes [17].

Starting from the original report of Seaquist et al. [18] of a familial aggregation of DN in T1D, several other lines of evidence have supported a strong genetic predisposition to the development of this complication. However, attempts to reveal the genetic architecture of DN have so far had inconclusive results, due to limited sample size, variable definition of the DN phenotype, and survival bias related to the development of this complication. The study by Sandholm et al. represents the largest GWAS for DN reported to date.

Two loci were associated with ESRD with a significant GWAS p level $<10^{-8}$: *AFF3* and an intergenic locus between *RGMA* and *MCTP2*. Experimental evidence for a role of *AFF3* in ESRD was provided by showing that its expression levels were related to TGF- β_1 -driven fibrosis in an epithelial cell culture model. Although these results are intriguing, they need to be accepted with caution given that the association between *AFF3* and ESRD was not replicated in all cohorts included in the meta-analysis.

Unfortunately, when DN was used as the outcome phenotype, the results were disappointing; no genome-wide signal was identified. The only relevant finding was a consistent association found across the cohorts for an intronic SNP in *ERBB4*, which encodes a member of the EGF receptor protein kinase family, and seems to modulate kidney tubule proliferation and polarity during nephrogenesis [19].

The disappointing results for DN could be due to the phenotype definition, relying on clinical criteria, which often vary from study to study. Although this is the largest GWAS for ESRD and DN in T1D, there is still a strong need for further studies to better define the genetic architecture of DN.

Food for thought

A new attempt to improve islet transplant survival

Alpha-1-antitrypsin reduces inflammation and enhances mouse pancreatic islet transplant survival

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Proc Natl Acad Sci USA 2012;109:15443–15448

Background: The promise of islet cell transplantation cannot be fully realized in the absence of improvements in engraftment of resilient islets.

Methods: The authors assessed the effects of α_1 -antitrypsin (AAT) in a syngeneic non-autoimmune islet graft model. A marginal number of syngeneic mouse islets were transplanted into non-autoimmune diabetic hosts. Islet function was analyzed in control and AAT-treated hosts.

Results: In controls, marginal mass islet transplants did not restore euglycemia. Outcomes were dramatically improved by short-term AAT treatment. Transcriptional profiling identified 1,184 differentially expressed transcripts in AAT-treated hosts at 3 days posttransplantation. Systems biology-based analysis revealed AAT downregulated regulatory hubs formed by inflammation-related molecules (e.g. TNF- α , NF- κ B). These results were confirmed by QRT-PCR and immunohistology.

Conclusions: These findings suggest that short-term AAT treatment of human islet transplant recipients may be worthy of a clinical trial.

An attractive topic in the context of T1D is islet cell transplantation, which could represent a definitive cure. However, as already highlighted in previous *Yearbook* chapters, there are still several problems limiting the application of islet transplantation, including the need of islets from more than one donor, prolonged immunosuppression therapies, and 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, over the years there have been major efforts to develop ways of improving islets survival.

The article by Koulmanda et al. represents a further step in this field, assessing the potential effect of treatment with ATT in a syngeneic non-autoimmune islet graft model.

The rationale for this approach is related to the anti-inflammatory and islet protective effect of ATT on pancreatic islets [20]. AAT-treated islet recipient mice became euglycemic short term and 70% remained euglycemic for at least 60 days. In contrast, among untreated mice, only 10% regained euglycemic state in the short term and none remained euglycemic at day 10. The systems biology approach showed that ATT suppressed inflammation and immune response genes at day 3, and network analysis confirmed that the downregulated molecules were implicated in inflammatory pathways. These results are intriguing and might lead to trials testing the value of treatment with ATT in humans.

Food for thought

Pancreas atrophy as a marker of subclinical T1D

Pancreas organ weight in individuals with disease-associated autoantibodies at risk for type 1 diabetes

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JAMA 2012;308:2337–2339

Background: Autopsy and imaging studies suggest that pancreatic volume is reduced in subjects with T1D. However, there is no information on when pancreas atrophy starts.

Methods: Pancreas weight was assessed in subjects at risk of T1D, in subjects with T1D and in controls. Pancreata were recovered from cadaveric multiorgan donors in the USA and considered for the study following consent from the next of kin. Prior to organ harvest, screening for GAD antibodies and insulinoma-associated 2ic was conducted. This information was examined with post-harvest data on insulin autoantibodies and zinc transporter 8 antigens. Information on demographic and medical history was obtained through chart review.

Results: The study population comprised 23 control subjects, 8 individuals without T1D who were positive for single islet autoantibodies, and 20 individuals with T1D. The three groups were comparable for age, sex distribution, and clinical characteristics. Pancreatic weight was significantly lower in both the single autoantibody group: 61.3 g (95% CI 46.8–75.8) and the T1D group: 44.9 g (95% CI 36.0–53.9; $p < 0.001$) compared to controls: 81.4 g (95% CI 73.0–89.8).

Conclusions: These results suggest that atrophy of the pancreas may be an early, subclinical feature of T1D, which might be used as a marker for disease risk assessment.

This research letter reports some very interesting preliminary results, suggesting that atrophy of the pancreas might be an indicator of subclinical T1D.

A previous study showed that pancreatic volume was reduced by 26% in patients with T1D within months of diagnosis, suggesting that atrophy begins years before the onset of clinical disease [21].

The present study represents a step forwards in the understanding of when pancreas atrophy occurs. The study finding of a progressive decrease of mean pancreatic weight from control subjects to those with autoantibodies and even more in those with T1D, suggests that pancreas atrophy is a phenomenon paralleling the development and progression of the disease.

The study presents limitations related to the small sample size, autoimmunity based on single autoantibody positivity and inability to distinguish between atrophy of the exocrine from that of the endocrine pancreatic tissue. Further studies are therefore required to confirm the present findings, which are of particular interest in view of developing ways and markers for predicting disease development and progression.

Reviews

Wide between-country variation in DKA frequency

Variation between countries in the frequency of diabetic ketoacidosis at first presentation of type 1 diabetes in children: a systematic review

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Diabetologia 2012;55:2878–2894

Background: Diabetic ketoacidosis (DKA) is a common complication of T1D, and is associated with both short-term risks and long-term consequences. The aim of this study was to assess the worldwide variation in presentation of T1D to inform future interventions to reduce this excess morbidity and mortality.

Methods: A systematic review was performed, assessing studies indexed on PubMed, EMBASE, Web of Science, Scopus or CINAHL before March 2011. The studies included unselected groups of children presenting with new-onset T1D and had to report the proportion of children presenting with DKA, as defined on measurement of pH or bicarbonate.

Results: 65 studies comprising over 29,000 children in 31 countries were included. The frequency of DKA at diagnosis ranged from 12.8 to 80%, with highest frequencies in the United Arab Emirates, Saudi Arabia and Romania, and the lowest in Sweden, the Slovak Republic and Canada. The frequency of DKA was inversely associated with gross domestic product, latitude and background incidence of T1D.

Conclusions: This systematic review shows for the first time a variation in the frequency of DKA at presentation of T1D in children across countries. This variation may be partly explained by different levels of disease awareness and healthcare provision.

DKA is a serious life-threatening complication, representing the leading cause of mortality in children with T1D [22]. A previous paper by this group identified risk factors for the development of DKA at first presentation of T1D, represented by younger age, diagnostic errors, ethnic minority status, lack of health insurance in the USA, lower body mass index, preceding infections, and delayed treatment [23]. In contrast, there are some protective factors, represented by having a first-degree relative with T1D at the time of diagnosis, higher parental education, and higher background incidence of T1D [23].

The novelty of this recent systematic review is the characterization of a wide variation, from 12.8 to 80%, in the frequency of DKA at diabetes onset across a large number of countries. Countries with the highest frequency were United Arab Emirates (80%), Romania (67%), Taiwan (65%) and Saudi Arabia (59%), whereas the lower frequencies were found in Sweden (14%), Canada (18.6%), Finland (22%) and Hungary (23%).

Interestingly, the frequency of DKA was inversely related to the incidence of T1D in the countries, suggesting that a higher incidence of the disease may lead to higher levels of awareness and an earlier diagnosis. However, this inverse association could be also due to different T1D phenotypes, so that in countries with lower incidences, the presentation might be more aggressive.

An inverse association with latitude, characterized by a progressive increase in DKA frequency with distance from the equator was also observed, and this could reflect differences in disease phenotypes or in the level of medical assistance or gross domestic product. In addition, a hotter weather in countries closer to the equator might represent a trigger for the metabolic derangements characterizing DKA.

Although the study findings have some limitations, such as the heterogeneity of the studies and the lack of data from several countries in Africa and Asia, they highlight the wide variation across countries, at least in part due to differences in awareness of this complication and differences in health-care provision. The latter two aspects could be improved and preventive strategies for DKA should be country-specific. Further studies are needed to identify additional reasons for the differences in DKA frequency.

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