Type 1 Diabetes: Clinical and Experimental

Francesco Chiarelli and Cosimo Giannini

Department of Paediatrics, University of Chieti, Chieti, Italy

Type 1 diabetes mellitus (T1D) is the most common endocrine disease in childhood and adolescence. The increasing worldwide incidence of diabetes highlights the increased need for new means of preventing or retarding the development and progression of diabetes and diabetes-related complications.

In particular, among the several research issues explored, four key fields offer the most promising perspectives: stem cells, offering the clinically opportunity of restoring the autoimmune-related depletion of the β -cells; genomic and proteomic, to define genetic findings as well as biomarkers able to characterize those subjects at increased risk of developing diabetes and diabetes-related complications, and immune-modulation aimed at preventing or reversing the immune-mediated mechanisms involved in diabetes. The continuous progress achieved by these main research areas suggests promising opportunities for better diagnosis and treatment of diabetes in the future.

Mechanism of the year

Curative and β cell regenerative effects of α_1 -antitrypsin treatment in autoimmune diabetic NOD mice

Koulmanda M, Bhasin M, Hoffman L, Fan Z, Qipo A, Shi H, Bonner-Weir S, Putheti P, Degauque N, Libermann TA, Auchincloss H Jr, Flier JS, Strom TB
Departments of Surgery and Medicine, Harvard Medical School, Boston, Mass., USA
strom@bidmc.harvard.edu

Proc Natl Acad Sci USA 2008;105:16242–16247

Background: Invasive insulinitis represents a destructive T-cell-dependent autoimmune process directed against insulin-producing β cells that is central to the pathogenesis of type 1 diabetes mellitus (T1D) in humans and in a non-obese diabetic (NOD) mouse model. Few therapies have succeeded in restoring long-term, drug-free euglycemia and immune tolerance to β cells in overtly diabetic NOD mice, and none have demonstrably enabled enlargement of the functional β -cell mass. The impact of inflammatory cytokines on the commitment of antigen-activated T cells to various effectors or regulatory T-cell phenotypes and insulin resistance and defective insulin signaling have been emphasized in recent studies.

Methods: In this study Koulmanda et al. tested the hypothesis that inflammatory mechanisms trigger insulinitis, insulin resistance, faulty insulin signaling, and the loss of immune tolerance to islets.

Results: In NOD mice, with recent-onset T1D, treatment with α_1 -antitrypsin (AAT), an agent that dampens inflammation, does not directly inhibit T-cell activation, ablates invasive insulinitis, and restores euglycemia, immune tolerance to β cells, normal insulin signaling, and insulin responsiveness through favorable changes in the inflammation milieu.

Conclusions: These results suggest that the functional mass of β cells expands in AAT-treated diabetic NOD mice.

Several therapeutic approaches for restoring either immune tolerance to β cells as well to the β -cell mass have been investigated during the last decades [1, 2]. Interestingly in this study Koulmanda et al. were able to demonstrate an effective role on autoimmune-induced β -cell loss achieved by treatment with AAT, an acute-phase reactant with serine proteinase inhibitor, and anti-inflammatory and anti-apoptotic effects. In fact in NOD mice with overt new onset T1D, AAT treatment favorably served to dampen expression of proinflammatory, but not anti-inflammatory, cytokines without affecting T cells and the acquisition of an activated phenotype. In addition, AAT therapy was able to decrease

the expression of T-helper 1 (Th1)-specific T-bet and Th17-specific retinoic acid-related orphan receptor- γ (ROR- γ t) transcription factors as well as *mo* Foxp3 expression. Therefore, AAT mainly acts by tilting either the balance of expression of proinflammatory to anti-inflammatory cytokines as well the balance of T cell Th1/Th17 effector (dramatically downregulated) to regulatory T-cell genes, sharply toward predominance of anti-inflammatory and regulatory T-cell gene expression. In addition, in the fat tissue of diabetic mice, AAT treatment was able to dampen and restore toward normal three inflammation-related molecules (TNF- α , IL-4, and NF- κ B) resulting in a reestablished insulin sensitivity and signaling in peripheral tissues.

These preliminary results open a new encouraging and exciting view on the characterization of the complex network of immune-system activation and cytokine/chemokine-mediated β -cell destruction. In particular, ATT therapy could add further power to previous similar studies (for instance those using anti-CD3 mAb) [3] with the aim of preserving β -cell mass and function. Therefore, further trials are needed in order to completely characterize the effect of ATT in β -cell depletion in young patients with T1D.

In vivo reprogramming of adult pancreatic exocrine cells to β cells

Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA

Department of Stem Cell and Regenerative Biology, Howard Hughes Medical Institute, Harvard Stem Cell Institute, Harvard University, Cambridge, Mass., USA

dmelton@harvard.edu

Nature 2008;455:627–632

Background: One goal of regenerative medicine is to instructively convert adult cells into other cell types for tissue repair and regeneration. Although isolated examples of adult cell reprogramming are known, there is no general understanding of how to turn one cell type into another in a controlled manner.

Methods/Results: Using a strategy of reexpressing key developmental regulators *in vivo*, Zhou et al. identified a specific combination of three transcription factors [Ngn3 (also known as Neurog3), Pdx1 and Mafa] that reprograms differentiated pancreatic exocrine cells in adult mice into cells that closely resemble β cells. Results showed that the induced β cells are indistinguishable from endogenous islet β cells in size, shape and ultrastructure. In addition, these cells expressed genes essential for β -cell function and could ameliorate hyperglycemia by remodeling local vasculature and secreting insulin.

Conclusions: These results provide an example of cellular reprogramming using defined factors in an adult organ and suggest a general paradigm for directing cell reprogramming without reversion to a pluripotent stem cell state.

In this study Zhou et al. have smartly proved the first and relevant step for the future application of regenerative medicine in the treatment of patients with T1D. By starting from the concept of cellular reprogramming or lineage reprogramming [4, 5] (a process characterized by the switching of cells of one type into another type), they investigate the complex and strict step-by-step activation of several transcription factor genes which lead to a mature β cell from a human embryonic stem (hES) cell [6]. After injecting a mixture of adenoviruses into the pancreata of 2-month-old adult mice (that coexpress 9 of the most relevant transcription factors) the investigators were able to demonstrate that the combination of only 3 (*Ngn3*, *Pdx1* and *Mafa*) of the transcription factors tested were able to reprogram a fully differentiated exocrine cell into cells that closely resemble β cells. In fact, new insulin-positive cells were detected at lower level 3 days after injection, but the intensity of insulin staining increased gradually so that by day 10 the level was comparable to that of endogenous β cells, and still present after 3 months. In addition, surprisingly Zhou et al. showed that the reprogramming of exocrine cells to β cells did not involve multiple rounds of cell proliferation and therefore conversion between exocrine and β cells may require fewer epigenetic changes. Unfortunately, they noticed that the induced β cells did not organize into islet structures and remained as single cells or small clusters. Undoubtedly, this represents a relevant limitation, as the lack of organization impairs their function. In addition, the adjustment of this process for more direct, abundant and easily accessible patient-specific human cells such as fibroblasts, blood cells or adipocytes are needed. Therefore, although it appears clearly evident that further additional studies are needed in order to completely characterize the process and to bypass the related limitations, Zhou et al. have shown the first step in a process that, in the near future, will hopefully enable patient-specific cell therapies by utilizing the cellular reprogramming concept.

CTLs are targeted to kill β cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope

Skowera A, Ellis RJ, Varela-Calviño R, Arif S, Huang GC, Van-Krinks C, Zaremba A, Rackham C, Allen JS, Tree TI, Zhao M, Dayan CM, Sewell AK, Unger W, Drijfhout JW, Ossendorp F, Roep BO, Peakman M

Department of Immunobiology, King's College London, London, UK

mark.peakman@kcl.ac.uk

J Clin Invest 2008;118:3390–3402

Background: The final pathway of β -cell destruction leading to insulin deficiency, hyperglycemia, and clinical T1D is not completely known.

Methods: In this study Skowera et al. showed that circulating CTLs can kill β cells via recognition of a glucose-regulated epitope.

Results: First, 2 naturally processed epitopes from the human preproinsulin signal peptide by elution from HLA-A2 (specifically, the protein encoded by the A*0201 allele) molecules were identified. Processing of these was unconventional, requiring neither the proteasome nor transporter-associated processing (TAP). However, both epitopes were major targets for circulating effector CD8+ T cells from HLA-A2+ patients with T1D. Moreover, cloned preproinsulin signal peptide-specific CD8+ T cells killed human β cells in vitro. Critically, at a high glucose concentration, β -cell presentation of preproinsulin signal epitope increased, as did CTL killing.

Conclusions: This study provides direct evidence that autoreactive CTLs are present in the circulation of patients with T1D and that they can kill human β cells. In addition, these results identify a mechanism of self-antigen presentation that is under pathophysiological regulation and could expose insulin-producing β cells to increasing cytotoxicity at the later stages of the development of clinical diabetes. Therefore, data suggest that autoreactive CTLs are important targets for immune-based interventions in T1D and argue for early, aggressive insulin treatment to preserve remaining β cells.

Skowera et al. add some relevant information on the immune-based mechanisms involved in the β -cell mass destruction and offer a potential explanation for the preservation on β cells achieved by early aggressive insulin therapy [7–9]. First, by creating surrogate β cells expressing a single autoantigen and HLA class I molecules, they were able to investigate their naturally displayed peptide repertoire. In fact, it was shown that surrogate β cells uncovered the signal peptide (SP) as a source of preproinsulin (PPI) epitopes that constitute major targets of CD8+ T cell responses in HLA-A2+ patients with T1D. In addition, in vitro analysis showed that human HLA-A2-expressing β cells are killed by expanded clones of PPI SP-specific CD8+ T cells, especially when β cells are exposed to high glucose concentrations. These findings offer three major novelties. First, these results suggest the possibility of developing CD8+ T-cell-specific strategies to halt β -cell destruction. In addition, in vitro analysis clearly showed that hyperglycemia-induced β -cell stress determines a characteristic and novel pathway through which PPI SP epitopes are expressed. This observation implies that I-CLiPs and ER proteases are critical for CTL epitope presentation. The relevant role of these epitopes in autoimmune destruction of β cells has been demonstrated in an interesting recent study. In fact, Toma et al. [10] were able to define class-I-restricted epitopes located within the leader sequence of human preproinsulin through in vivo (transgenic mice) and ex vivo (diabetic patients) assays, confirming the possible role of preproinsulin-specific CD8+ T cells in human T1D. Targeting these pathways could offer new therapeutic agents for prevention of β -cell destruction. Furthermore, these findings attempt to provide evidence of a mechanism leading β cells, stressed by the need to control blood glucose levels as diabetes develops, to become enhanced targets for the immune system. This observation suggests that an early and aggressive introduction of insulin treatment might be a possible rationale approach that could at least slow the progression of β -cell destruction. Previous studies, albeit on small numbers of subjects, have suggested that preservation of endogenous insulin reserve might benefit from administration of insulin in the pre-diabetic period [11] or from tight metabolic control soon after diagnosis [12, 13]. In contrast, other studies failed to prove a positive effect on diabetes prevention or delay by administering insulin in persons at high risk of diabetes [7, 8]. However, as there is a progressive loss of β -cell mass and function from the initiation of insulinitis through diabetes presentation, it could be speculated that the time at which the progression of disease is identified and treated is of

practical importance. In fact, the residual cells may represent the source of new β cells and combination agents that are more likely to be effective if there are β cells that can respond. Therefore, based on these findings further studies are needed to completely define the effectiveness of insulin alone or combined agents in the prevention of or slowing the progression of β -cell destruction.

New hope

Urinary proteomics in diabetes and chronic kidney diseases

Rossing K, Mischak H, Dakna M, Zürlig P, Novak J, Julian BA, Good DM, Coon JJ, Tarnow L, Rossing P
Steno Diabetes Center, Gentofte, Denmark
krossing@dadlnet.dk

J Am Soc Nephrol 2008;19:1283–1290

Background: Urinary biomarkers for diabetes, diabetic nephropathy, and non-diabetic proteinuric renal diseases were sought.

Methods: Using high-resolution capillary electrophoresis coupled with electrospray-ionization mass spectrometry, biomarkers were defined and validated in blinded data sets including 305 individuals.

Results: A panel of 40 biomarkers distinguished patients with diabetes from healthy individuals with 89% sensitivity and 91% specificity. In patients with diabetes, 102 urinary biomarkers differed significantly between patients with normo-albuminuria and nephropathy, and a model that included 65 of these correctly identified diabetic nephropathy with 97% sensitivity and specificity. Furthermore, this panel of biomarkers identified patients who had microalbuminuria and diabetes and progressed toward overt diabetic nephropathy over 3 years. Differentiation between diabetic nephropathy and other chronic renal diseases reached 81% sensitivity and 91% specificity. Many of the biomarkers were fragments of collagen type I, and quantities were reduced in patients with diabetes or diabetic nephropathy.

Conclusions: These data clearly show that analysis of the urinary proteome may allow early detection of diabetic nephropathy and may provide prognostic information.

Rossing et al. were able to detect a highly sensitive and specific urinary proteomic pattern distinct for diabetes, diabetic nephropathy, and non-diabetic proteinuric renal diseases. In fact, by analyzing urinary profiles with the online combination of capillary electrophoresis and electrospray mass spectrometry, they investigated whether a single or a combination of polypeptides, present at a frequency of >50%, may characterize patients with diabetes and their risk of developing vascular complications. In particular, after identifying a sequence of 40 identified biomarkers, they showed that 53 of 59 patients with diabetes (patients with diabetes and normo-albuminuria or microalbuminuria or nephropathy) and 32 of 35 healthy control subjects were correctly classified, resulting in 89% sensitivity and 91% specificity. The possibility of proteomic analysis to differentiate the severity of kidney damage (defined as normo- or microalbuminuria and as overt nephropathy) was further proven. In fact, after defining 65 of 102 identified biomarkers by a 'take-one-out' analysis, they showed 97% sensitivity and specificity reached by the linear combination of these polypeptides. In addition, more information was obtained by performing the analysis in 8 of 30 patients with diabetes and microalbuminuria which showed an increase in albuminuria of about 25% or progressed to macroalbuminuria during a 3-year follow-up interval. Thus, biomarkers were useful not only to detect patients with overt nephropathy but also to predict its development in patients with diabetes and microalbuminuria. This study clearly shows the contribution of system biology in the study of diabetes. In fact, a new generation of mass spectrometers will be available, with better sensitivity, mass accuracy and resolution; these new systems could offer new markers of kidney function. In addition, the most striking observation was the decreased excretion of specific collagen fragments in patients with diabetes and healthy controls as well as within patients with diabetes. Nonetheless, Rossing et al. showed several additional collagen fragments which were less common in patients with diabetic nephropathy compared with normo-albuminuric diabetic patients. As these fragments are likely products of specific proteases and are related to the balanced synthesis and activity of pro-

tease inhibitors, the complete characterization of the products might give relevant tools for further characterization of the natural history of diabetic nephropathy. In particular, they might serve as indicators of the activity of these enzymes as well as the synthesis of the proteins involved in the morphological kidney changes in diabetic nephropathy.

Tyrosine kinase inhibitors reverse type 1 diabetes in nonobese diabetic mice

Louvet C, Szot GL, Lang J, Lee MR, Martinier N, Bollag G, Zhu S, Weiss A, Bluestone JA

Diabetes Center and the Department of Medicine, University of California, San Francisco, Calif., USA

aweiss@medicine.ucsf.edu

Proc Natl Acad Sci USA 2008;105:18895–18900

Background: The recent development of small-molecule tyrosine kinase inhibitors offers increasing opportunities for the treatment of autoimmune diseases.

Methods: In this study, Louvet et al. attempted to investigate the potential of this new class of drugs to treat and cure T1D in the NOD mouse.

Results: Treatment of pre-diabetic and new onset diabetic mice with imatinib (Gleevec) prevented and reversed T1D. Similar results were observed with sunitinib (Sutent), an additional approved multi-kinase inhibitor, suggesting that the primary target of imatinib, c-Abl, was not essential in blocking the disease in this model. Additional studies with another tyrosine kinase inhibitor, PLX647 (targeting c-Kit and c-Fms) or an anti-c-Kit mAb showed only marginal efficacy, whereas a soluble form of platelet-derived growth factor receptor (PDGFR), PDGFR- β -Ig, rapidly reversed diabetes. These findings strongly suggest that inhibition of PDGFR is critical for reversing diabetes and highlight the crucial role of inflammation in the development of T1D. These conclusions were supported by the finding that the adaptive immune system was not significantly affected by imatinib treatment. Finally, and most significantly, imatinib treatment led to durable remission after discontinuation of therapy at 10 weeks in a majority of mice.

Conclusions: Long-term efficacy and tolerance is likely to depend on inhibiting a combination of tyrosine kinases supporting the use of selective kinase inhibitors as a new and potentially very attractive approach for the treatment of T1D.

In this promising study, starting from the demonstration of a positive effect of imatinib (an inhibitor of the inactive conformation of Abl protein tyrosine kinases) on several autoimmune diseases [14] and Crohn's disease [15], Louvet et al. investigated whether this drug might be effective in preventing or treating T1D. In particular, by treating the NOD mouse (a model autoimmune diabetes in which the disease occurs spontaneously at about 12–14 weeks and shares many phenotypic and genetic similarities with T1D in human subjects), they were able to demonstrate that tyrosine kinase inhibitors not only prevent the onset of diabetes, given during a pre-diabetic stage, but also reverse the disease when given at the time of diabetes presentation. Surprisingly, Louvet et al. showed that limiting treatment to 8–10 weeks, after the onset of diabetes, was sufficient to reverse the disease and induce long-term remission consistent with reestablishment of immune tolerance. However, it is relevant to stress that imatinib, while preventing clinical disease, diminishes but does not eliminate leukocyte infiltration of the pancreas. These results are consistent with recent studies demonstrating a direct protective effect of imatinib on type 2 diabetes in rodents [16] and suggest that this molecule and other kinase inhibitors (such as sunitinib) have a potential therapeutic effect in patients with T1D. Nevertheless, a complete characterization of the mechanisms by which these drugs act in autoimmune diseases need to be determined; in fact, whether imatinib operates through direct function on B or T cells or both, or indirectly by modulating the expression of INF- γ in T cells or of other chemokines or cytokines involved in β -cell loss is not completely defined. Therefore, newer studies evaluating the underlying mechanisms by which tyrosine kinase inhibitors stably prevent and reverse the immune-mediated response to self-cells are needed. Further findings will possibly make these drugs, or especially newer highly selective kinase inhibitors, realistic new therapeutic tools for clinical treatment of diabetes and other autoimmune diseases.

Young women with type 1 diabetes have lower bone mineral density that persists over time

Mastrandrea LD, Wactawski-Wende J, Donahue RP, Hovey KM, Clark A, Quattrin T

Department of Pediatrics, University at Buffalo, School of Medicine and Biomedical Sciences, Buffalo, N.Y., USA

ldm@buffalo.edu

Diabetes Care 2008;31:1729–1735

Background: Individuals with T1D have decreased bone mineral density (BMD); yet the natural history and pathogenesis of osteopenia are unclear. In a previous study Mastrandrea et al. showed that women with T1D (aged 13–35 years) have lower BMD than community age-matched healthy control subjects. In order to determine the natural history of BMD in young women with and without diabetes, they performed a 2-year follow-up BMD data of the previously selected cohort.

Methods: To estimate BMD dual-energy X-ray absorptiometry was used at baseline and 2 years later in 63 women with T1D and in 85 age-matched control subjects. HbA1C, IGF-1, IGF-binding protein-3, serum osteocalcin, and urine N-telopeptide were also measured at follow-up.

Results: After adjusting for age, BMI and oral contraceptive use, BMD at year 2 persisted to be lower in women ≥ 20 years of age with T1D compared with control subjects at the total hip, femoral neck, and whole body. Lower BMD values were observed in cases < 20 years of age compared with control subjects; however, the differences were not statistically significant. Lower BMD did not correlate with diabetes control, growth factors, or metabolic bone markers.

Conclusions: This study confirms lower BMD in young women with T1D compared to control subjects which appears to persist over time, particularly in women ≥ 20 years of age. Persistence of low BMD as well as failure to accrue bone density after age 20 years may contribute to the increased incidence of osteoporotic hip fractures documented in postmenopausal women with T1D.

Some studies have investigated the effects of diabetes-related metabolic and hormonal alterations on bone metabolism [17, 18]. However, very few studies have attempted to longitudinally characterize the natural history of BMD in patients with T1D. In this study, by performing a 2-year follow-up analysis, Mastrandrea et al. confirmed the lower BMD in the femoral neck and lateral spine in patients with T1D, especially in those older than 20 years. Furthermore, they documented that reduced values persisted during follow-up and that reduced BMD was associated with lower IGF1 levels in younger patients. Although this study has several limitations (the excessive number of women followed up compared to men, and the possible bias related to the selection of the patients enrolled) the persistently reduced BMD values during the follow-up clearly demonstrate that a complete and systematic characterization of bone mineral acquisition or turnover might be routinely adopted in all patients with T1D, especially during childhood and adolescence. Furthermore, better characterization of the exact mechanisms involved in the bone homeostasis in patients with T1D is needed. In fact, osteoporosis represent the most significant metabolic bone disease in patients with diabetes and is associated with an increased risk of the osteoporosis-related complications including hip fracture [19]. In particular, the characterization of markers of impaired bone homeostasis, able to identify patients at increased risk, are needed to use all the available preventive and therapeutic means in the daily care of patients with T1D.

A1C variability and the risk of microvascular complications in type 1 diabetes: data from the diabetes control and complications trial

Kilpatrick ES, Rigby AS, Atkin SL

Department of Clinical Biochemistry, Hull Royal Infirmary, Hull, UK

eric.kilpatrick@hey.nhs.uk

Diabetes Care 2008;31:2198–2202

Background: Debate remains as to whether short- or long-term glycemic instability confers an increased risk of microvascular complications in addition to that predicted by means of glycemia alone. In this study, Kilpatrick et al. analyzed data from the Diabetes Control and Complications Trial (DCCT) in order to assess whether HbA1c variability might influence the risk of retinopathy and nephropathy in patients with T1D.

Methods: During the DCCT HbA1c was collected quarterly in 1,441 individuals. The mean HbA1c and the SD of HbA1c variability after stabilization of glycemia (from 6 months onwards) were compared with the risk of retinopathy and nephropathy with adjustments for age, sex, disease duration, treatment group, and baseline HbA1c.

Results: By a multivariate Cox regression it was shown that the variability in HbA1c added to mean HbA1c in predicting the risk of development or progression of both retinopathy (hazard ratio 2.26 for every 1% increase in HbA1c SD [95% CI 1.63–3.14], $p < 0.0001$) and nephropathy (1.80 [1.37–2.42], $p < 0.0001$), in particular in conventionally treated patients.

Conclusions: This study documented that variability in HbA1c adds to the mean value in predicting microvascular complications in T1D. Thus, in contrast to analyses of DCCT data investigating the effect of short-term glucose instability on complication risk, longer-term fluctuations in glycemia seem to contribute to the development of retinopathy and nephropathy in patients with T1D.

In this study Kilpatrick et al. were able to confirm the role of HbA1c variability in the risk of diabetes complications. In fact, by using publicly accessible datasets collected by the DCCT (a 9-year randomized-controlled, follow-up study of 1,441 participants with T1D comparing the effect of intensive versus conventional insulin treatment on the risk of development of the microvascular complications) [20], they showed that the higher the HbA1c variability the greater the risk of developing both retinopathy and nephropathy. The risk was shown in the DCCT cohort overall and was also an individual feature of both treatment groups. In addition, the role of HbA1c variability was indirectly confirmed by the demonstration of a most pronounced effect among conventionally treated patients presumably due to the event rate or the range of variability or the spread of variability at any given mean HbA1c, which was larger than those for intensively treated patients. Overall, Kilpatrick et al. showed that the magnitude of the effect of HbA1c variability is marked, such that a 1% absolute increase in HbA1c-SD results in at least a doubling of retinopathy and an 80% increase in nephropathy risk. These findings are in contrast to those of a previous analysis of the DCCT data [21], which suggested that HbA1c variability had little effect on the risk of complications. However, in the previous study findings were probably distorted by the inclusion of all patient visits, even those visits before HbA1c stabilized at 6 months in intensively treated patients.

For clinicians involved in the care of patients with T1D it is essential to fully understand the relevant role of glycemic variability in the risk of developing micro- and macrovascular complications. In this perspective, recent technological and pharmacological advances have focused on reducing especially postprandial hyperglycemia which represents the main determinant of the increased glucose variability. Data from Kilpatrick et al. clearly show the importance of glucose variability and confirm data from the Pittsburgh Epidemiology Study which showed that HbA1c is an additional risk factor for the development of macrovascular complications [22]. In addition, although HbA1c is well known to represent the main predictor of diabetes-related complications, several studies have demonstrated a relevant role of postprandial and fasting hyperglycemia in metabolic control and diabetes complications. In fact, in patients with diabetes over quintiles of HbA1c both postprandial and fasting hyperglycemia are independent factors to be considered in order to reach the target of HbA1c and to reduce the risk of cardiovascular diseases [23, 24]. Thus, several postprandial events combine to make waves that may eventually wear down the endothelial mole. Similarly, instable metabolic control, as

defined by HbA1c fluctuation, may act like a strong wind causing billows that strike the wall and may rapidly tear it down, amplifying the increased risk associated with impaired metabolic control. In order to reduce the risk of diabetes-related complications, patients and the diabetic team should cooperate to maintain not only HbA1c levels and glucose fluctuations as low as possible and but also to minimize HbA1c variability as little as possible.

Important for clinical practice

Institution of basal-bolus therapy at diagnosis for children with type 1 diabetes mellitus

Adhikari S, Adams-Huet B, Wang YC, Marks JF, White PC

Division of Pediatric Endocrinology, Department of Dietary Behavior, University of Texas Southwestern Medical Center, Dallas, Tex., USA

soumya.adhikari@childrens.com

Pediatrics 2009;123:e673–678

Background: Adhikari et al. evaluated whether the institution of basal-bolus therapy immediately after diagnosis improved glycemic control in the first year after diagnosis for children with newly diagnosed T1D.

Methods: The charts of 459 children ≥ 6 years of age who were diagnosed as having T1D between July 1, 2002, and June 30, 2006 (212 treated with basal-bolus therapy and 247 treated with a more-conventional neutral protamine Hagedorn regimen) were reviewed. Furthermore, Adhikari et al. analyzed data obtained at diagnosis and at quarterly clinic visits and compared groups by using repeated-measures, mixed-linear model analysis. The records of 198 children with preexisting T1D of >1 -year duration who changed from the neutral protamine Hagedorn regimen to a basal-bolus regimen during the review period were also evaluated.

Results: Glargine-treated subjects with newly diagnosed diabetes had lower HbA1c levels 3, 6, 9, and 12 months after diagnosis than did neutral protamine Hagedorn-treated subjects (average HbA1c levels of 7.05% with glargine and 7.63% with neutral protamine Hagedorn, estimated across months 3, 6, 9, and 12, according to repeated-measures models adjusted for age at diagnosis and baseline HbA1c levels). Children with long-standing diabetes had no clinically important changes in their HbA1c levels in the first year after changing regimens.

Conclusions: Basal-bolus therapy with insulin glargine starting from the time of diagnosis of T1D was associated with improved glycemic control, in comparison with more-conventional neutral protamine Hagedorn regimens, during the first year after diagnosis.

This retrospective and large study reported by Adhikari et al. adds relevant and additional findings to the complete definition of the safety and effectiveness, and especially on the ability to achieve better glycemic control reached by newer insulin analogs. In fact, by comparing data obtained in a total of 459 children older than 6 years of age and diagnosed as having T1DM, they showed a significant association on improved glycemic control obtained with a basal-bolus treatment (with glargine), used from the onset of diabetes. In particular, they reported that this treatment results in 0.58% lower HbA1c level compared to a scheme with neutral protamine Hagedorn. The effects on glycemic control were shown to be particularly relevant in older patients. In fact, after categorizing treatment responses according to age (dichotomized at 10.5 years), a greater treatment effect was observed for the oldest group (with a HbA1c difference of 0.86 vs. 0.37%). In contrast, Adhikari et al. were not able to demonstrate similar effects in those patients (198 children with longstanding T1D diagnosed at >6 years of age) who changed from neutral protamine Hagedorn to glargine regimen.

Over the last decades the availability of newer insulin analogs has been shown to represent an important means aimed at improving metabolic control and quality of life [25]. Although several studies have demonstrated a significant improvement in glycemic control in children and adults by switching from neutral protamine Hagedorn to long-acting analogs [26], no data are available on the role of these new insulins used from the onset of T1D. In addition, these data introduce a relevant and

already opened question. In fact improved glycemic control is well known to be associated with a greater duration of endogenous insulin secretory capacity [9, 13]. On the other hand preservation of β -cell mass determines better metabolic control resulting in a lower risk of diabetes-related complications [27, 28]. These effects are particularly relevant in adolescent patients in whom the pubertal-related effects negatively influence metabolic control. Although promising, there are relevant limitations, in particular the study design, in the data reported by Adhikiri et al. Therefore, prospective, randomized, controlled trials are needed with the aim of determining whether early institution of basal-bolus therapy with newer insulin analogs, started from the time of diagnosis of T1D, might positively affect β -cell mass.

Clinical trials, new treatments

GAD treatment and insulin secretion in recent-onset type 1 diabetes

Ludvigsson J, Faresjö M, Hjorth M, Axelsson S, Chéramy M, Pihl M, Vaarala O, Forsander G, Ivarsson S, Johansson C, Lindh A, Nilsson NO, Aman J, Ortqvist E, Zerhouni P, Casas R

Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden

johnny.ludvigsson@lio.se

N Engl J Med 2008;359:1909–1920

Background: The 65-kD isoform of glutamic acid decarboxylase (GAD) is a major auto-antigen in patients with T1D. In this study the ability of alum-formulated GAD (GAD-alum) to reverse recent-onset T1D in patients 10 to 18 years of age was assessed.

Methods: Seventy young patients with T1D who had fasting C-peptide levels above 0.1 nmol/l (0.3 ng/ml) and GAD autoantibodies, recruited within 18 months after receiving the diagnosis of diabetes, were randomly assigned to receive subcutaneous injections of 20 μ g of GAD-alum (35 patients) or placebo (alum alone, 35 patients) on study days 1 and 30. At day 1 and months 3, 9, 15, 21, and 30, a mixed-meal tolerance test was performed in all patients in order to stimulate residual insulin secretion (measured as the C-peptide level). The effect of GAD-alum on the immune system was also studied.

Results: Insulin secretion gradually decreased in both study groups. The study group had no significant effect on change in fasting C-peptide level after 15 months (the primary end point) compared to controls. Fasting C-peptide levels declined from baseline levels to significantly less over 30 months in the GAD-alum group than in the placebo group (-0.21 vs. -0.27 nmol/l [-0.62 vs. -0.81 ng/ml], $p = 0.045$), as did stimulated secretion measured as the area under the curve (-0.72 vs. -1.02 nmol/l/2 h [-2.20 vs. -3.08 ng/ml/2 h], $p = 0.04$). No protective effect was seen in patients treated 6 months or more after receiving the diagnosis. Adverse events appeared to be mild and similar in frequency between the 2 groups. The GAD-alum treatment induced a GAD-specific immune response.

Conclusions: GAD-alum may contribute to the preservation of residual insulin secretion in patients with recent-onset T1D, although insulin requirement is not modified 30 months after onset of clinical diabetes.

In this longitudinal, multicenter, placebo-controlled, randomized and relatively large study, Ludvigsson et al. were able to investigate whether subcutaneous administration of the 65-kD isoform of GAD, one of the major autoantigens in patients with T1D, would reduce or halt the loss of residual insulin secretion. Firstly, in this clinical trial two consecutive subcutaneous administrations of a recombinant human GAD in a standard vaccine formulation (a primary injection and a booster injection of 20 μ g each) have been well tolerated. In fact, no relevant adverse events were documented during the entire study follow-up. In addition, a gradual loss of β -cell function was documented in both study groups as indicated by the progressive decrease from the baseline level in both fasting and stimulated C-peptide secretion. Furthermore, GAD-alum treatment was not associated with a change in fasting C-peptide level between baseline and month 15. At variance, in the GAD-alum group fasting as well as stimulated C-peptide levels showed a significantly smaller decline after 30 months, compared to the placebo group. However, an apparent protective effect of the GAD-alum treatment

on C-peptide secretion was demonstrated only in patients treated for less than 6 months after diagnosis. In contrast, no effects on insulin requirement were documented by GAD-alum treatment. Preservation of β -cell mass represents a relevant opportunity in patients with T1D [28]. In fact, it is well documented that even modest residual insulin secretion, with stimulated C-peptide levels of >0.2 nmol/l (0.6 ng/ml), has been reported to provide clinically meaningful benefits in terms of reducing long-term complications [27]. Previous studies and especially those with anti-CD3 monoclonal treatment [3] have shown minimal benefits which appear to be associated with relatively relevant adverse effects. The relatively safety and the similar duration and magnitude of the GAD-alum treatment encourage large-scale confirmatory studies with GAD-alum. In addition, further studies are needed to characterize the immunological mechanisms implicated in the GAD-alum-mediated modulation of autoimmunity. These results will provide further help to understand immune-mediated depletion of β cells in patients with T1D.

Effects of the selective serotonin reuptake inhibitor fluoxetine on counterregulatory responses to hypoglycemia in individuals with type 1 diabetes

Briscoe VJ, Ertl AC, Tate DB, Davis SN

Department of Medicine, Vanderbilt University, Nashville, Tenn., USA

steve.davis@vanderbilt.edu

Diabetes 2008;57:3315–3322

Background: Chronic administration of the serotonin reuptake inhibitor (SSRI) fluoxetine has been shown to augment counter-regulatory responses to hypoglycemia in healthy humans. However, virtually no information exists regarding the effects of fluoxetine on integrated physiological counter-regulatory responses during hypoglycemia in T1D. Thus, the specific aim of this study was to test whether in individuals with T1D 6-week use of the SSRI fluoxetine would amplify autonomic nervous system counter-regulatory responses to hypoglycemia.

Methods: 18 patients with T1D (14 men/4 women aged 19–48 years with BMI 25 ± 3 and HbA1C $7.0 \pm 0.4\%$) participated in randomized, double-blind 2-hour hyperinsulinemic ($9 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)-hypoglycemic clamp studies before and after 6 weeks of fluoxetine administration ($n = 8$) or identical placebo ($n = 10$). Glucose kinetics was determined by 3-tritiated glucose. Muscle sympathetic nerve activity was determined by microneurography.

Results: Hypoglycemia (2.8 ± 0.1 mmol/l) and insulinemia (646 ± 52 pmol/l) were similar during all clamp studies. Autonomic nervous system, neuroendocrine, and metabolic counter-regulatory responses remained unchanged in the placebo group. However, the key autonomic nervous system (epinephrine, norepinephrine, and muscle sympathetic nerve activity), metabolic (endogenous glucose production and lipolysis), and cardiovascular (systolic blood pressure) counter-regulatory responses during hypoglycemia were significantly increased by fluoxetine administration ($p < 0.05$).

Conclusions: Thus, 6-week administration of the SSRI fluoxetine can amplify the autonomic nervous system and metabolic counter-regulatory mechanisms during moderate hypoglycemia in patients with T1D. Consequently, the use of fluoxetine may be helpful in increasing epinephrine responses during hypoglycemia in clinical practice.

In this randomized, double-blind study Briscoe et al. were able to explore the effects on the autonomic nervous system, as well as the neuroendocrine and metabolic counter-regulatory mechanisms during hypoglycemia in patients with T1D before and after 6 weeks of fluoxetine (a selective serotonin reuptake inhibitor) compared to placebo administration. In particular, by using a 2-hour hyperinsulinemic-hypoglycemic clamp, they showed that a period of 6 weeks of high-dose fluoxetine significantly increases sympathetic nervous system, hypothalamic-pituitary-adrenal pathways, and metabolic (endogenous glucose production, lipolysis with increased glycerol and non-esterified fatty acids) counter-regulatory responses during hypoglycemia in patients with long-duration T1D. Interestingly, the most notable finding from the present study was the striking increase in epinephrine responses during hypoglycemia following fluoxetine. Despite equivalent insulin and glucose levels during the hypoglycemic clamps, fluoxetine resulted in a 90% increase in epinephrine levels. These findings are of great relevance in patients with T1D and a long diabetes duration. In fact, as the glucagon response to hypoglycemia is lost in patients with T1D with increasing disease duration

[29], epinephrine becomes the critical counter-regulatory hormone against acute hypoglycemia. Accompanying the amplified sympathetic nervous system responses were significant increases in blood pressure and heart rate during hypoglycemia following fluoxetine. Fluoxetine only amplified counter-regulatory responses during hypoglycemia and did not increase basal homeostatic mechanisms. Thus, there were no differences in baseline cardiovascular, metabolic, and neuroendocrine parameters following fluoxetine or placebo.

The reported effects of fluoxetine on physiological responses during hypoglycemia in a group of metabolically well-controlled young patients with T1D represents an important new perspective in the daily care of patients with diabetes. In fact, it is well known that hypoglycemia remains the major barrier to even near normalization of glucose in patients with T1D, especially in childhood [29]. Furthermore, patients with recurrent hypoglycemic episodes experience the dangerous 'hypoglycemia unawareness'. However, the dose of fluoxetine used in this study is higher than the average dose of the drug used in clinical practice [30] and therefore the similar effect of lower doses must be proven. In addition, the mechanisms of fluoxetine activity is not completely defined. Activation of a number of serotonergic receptors (5HT1A, 5HT1C, 5HT2, and 5HT3) has been demonstrated to increase autonomic nervous system outflow. Additionally, it is not defined whether the fluoxetine effect was being sensed at central (i.e., brain), peripheral (i.e., adrenal gland), or even both sites [31]. Therefore, it is important to perform further studies in order to thoroughly define the activity of selective serotonin reuptake inhibitors in improving counter-regulatory response to hypoglycemia in young patients with T1D.

New mechanisms

Selective death of autoreactive T cells in human diabetes by TNF or TNF receptor 2 agonism

Ban L, Zhang J, Wang L, Kuhlreiber W, Burger D, Faustman DL

Department of Immunobiology, Massachusetts General Hospital and Harvard Medical School, Boston, Mass., USA

faustuman@helix.mgh.harvard.edu

Proc Natl Acad Sci USA 2008;10:13644–13649

Background: As the immunosuppressive drugs are nonspecific, produce high levels of adverse effects, and are not based on mechanistic understanding of disease, their real application in human autoimmune diseases is limited. Destroying the rare autoreactive T lymphocytes causing autoimmune diseases would improve treatment. In animal models, TNF selectively kills autoreactive T cells, thereby hampering disease onset or progression. Here, Ban et al. attempted to determine, in fresh human blood, whether TNF or agonists of TNF selectively kill autoreactive T cells, while sparing normal T cells.

Methods: They isolated highly pure CD4 or CD8 T cells from patients with T1D (n = 675), other autoimmune diseases, and healthy controls (n = 512).

Results: By using a two cell death assays, they found that a subpopulation of CD8, but not CD4, T cells in patients' blood was vulnerable to TNF or TNF agonist-induced death. One agonist for the TNFR2 receptor exhibited a dose-response pattern of killing. In T1D, the subpopulation of T cells susceptible to TNF or TNFR2 agonist-induced death was traced specifically to autoreactive T cells to insulin, a known autoantigen. In addition, other activated and memory T-cell populations were resistant to TNF-triggered death.

Conclusions: Therefore, autoreactive T cells, although rare, can be selectively destroyed in isolated human blood. TNF and a TNFR2 agonist may offer highly targeted therapies, with the latter likely to be less systemically toxic.

In this in vitro study, the TNF's role as a selective killer of autoreactive CD8 T cells in humans was investigated. By exposing T cells (isolated from the blood specimens of patients with T1D as well as other autoimmune diseases and normal control subjects) to TNF, and agonists that mimicked TNF's function (TNF-receptor-1 [TNFR1] or TNF-receptor-2 [TNFR2]), they showed that TNF exposure kills a subset of human CD8 T cells from patients with T1D and other autoimmune diseases without any

effect on CD4 T cells. In addition Ban et al. demonstrated that the death of this subpopulation of CD8 T cells was also triggered with a specific agonist for TNFR2 that mimics TNF's actions. In contrast, TNFR1 agonists did not trigger cell death of diabetic autoreactive T cells. Furthermore, these results showed that, in specimens from patients with T1D, a subpopulation of insulin autoreactive CD8 T cells specific for the HLA class I insulin-fragment died upon exposure to a TNFR2 agonist, confirming that the TNF pathway could be a target for new types of treatments. In contrast, activated CD8 T cells in diabetics to non-autoreactive peptides such as directed to CMV or EBV viral fragments, were resistant to TNF agonism, confirming the specificity of the TNF pathway as a targeted method of killing only autoreactive CD8 T cells. The capacity of a TNFR2 agonist to kill autoreactive diabetic CD8 T cells has relevant therapeutic implications for drug safety as potential therapeutic opportunity might be associated to minimal toxicity. Destruction of rare autoreactive T cells in autoimmune diseases represents an elusive therapeutic goal designed to produce marked benefit over current treatments that are nonspecific and plagued by adverse effects. A defective TNF signaling pathway, which leads to cell death, now provides, at least in vitro, a unique opportunity in human autoimmune diseases to kill only autoreactive T cells. The continuous progress in knowledge of the immunological mechanisms inducing the β -cell loss as well as the newer cytokines involved in immune system regulation, in addition to the newer formulation of highly selective drugs, will possibly offer new potential therapeutic opportunity in patients with T1D.

A microsphere-based vaccine prevents and reverses new-onset autoimmune diabetes

Phillips B, Nylander K, Harnaha J, Machen J, Lakomy R, Styche A, Gillis K, Brown L, Lafreniere D, Gallo M, Knox J, Hogeland K, Trucco M, Giannoukakis N

Diabetes Institute, Division of Immunogenetics, Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, Penn., USA

ngiann1@pitt.edu

Diabetes 2008;57:1544–1555

Background: The aim of the study was to verify whether antisense oligonucleotide-formulated microspheres are efficacious to prevent T1D and to reverse new-onset disease.

Methods: Microspheres carrying antisense oligonucleotides to CD40, CD80, and CD86 were delivered into NOD mice. Glycemia was monitored to determine disease prevention and reversal. In recipients that remained and/or became diabetes free, spleen and lymph node T cells were enriched to determine the prevalence of Foxp3(+) putative regulatory T-cells (Treg cells). Splenocytes from diabetes-free microsphere-treated recipients were adoptively co-transferred with splenocytes from diabetic NOD mice into NOD-scid recipients. Live-animal in vivo imaging measured the microsphere accumulation pattern. To rule out nonspecific systemic immunosuppression, splenocytes from successfully treated recipients were pulsed with β -cell antigen or ovalbumin or co-cultured with allogenic splenocytes.

Results: T1D was prevented by microsphere adoption and, most importantly, the system exhibited a capacity to reverse clinical hyperglycemia, suggesting reversal of new-onset disease. The microspheres augmented Foxp3(+) Treg cells and induced hyporesponsiveness to NOD-derived pancreatic β -cell antigen, without compromising global immune responses to alloantigens and nominal antigens. T cells from successfully treated mice suppressed adoptive transfer of disease by diabetogenic splenocytes into secondary immunodeficient recipients. Finally, microspheres accumulated within the pancreas and the spleen after either intraperitoneal or subcutaneous injection. Dendritic cells from the spleens of microsphere-treated mice exhibit decreased cell surface CD40, CD80, and CD86.

Conclusions: This novel microsphere formulation represents the first diabetes-suppressive and reversing nucleic acid vaccine that confers an immunoregulatory phenotype to endogenous dendritic cells.

Starting from the possibility to confer functional immaturity (defined by the capacity to activate and maintain immunoregulatory, 'suppressive' cell networks) to the dendritic cells (using systemic and molecule-specific approaches) [32], Phillips et al. evaluated whether antisense oligonucleotide-formulated microspheres are able to prevent T1D and to reverse new-onset disease. In particular, by using a PROMAXX microsphere delivery system (which has been demonstrated to be neutral with respect to dendritic cell maturation state in vivo) they formulated a PROMAXX-microsphere-based vaccine characterized by a mixture of the CD40, CD80, and CD86 antisense oligonucleotides (AS-MSPs).

Thereafter, the microspheres with AS-MSPs or with scrambled control sequences (SCR-MSP) were injected subcutaneously or intraperitoneally at a site anatomically proximal to the pancreatic lymph nodes, into 5- to 8-week-old NOD female mice. Thus they showed that one single injection of AS-MSP, at a site anatomically proximal to the pancreatic lymph nodes, significantly delayed onset of diabetes, and 8 consecutive injections were very efficacious in preventing the disease altogether. Similarly, they showed that injections performed soon after diabetes development were able to reverse diabetes. Furthermore, AS-MSP treatment was shown to increase the prevalence of Foxp3⁺ CD25⁺ putative regulatory T cells in vivo and to yield T cells hypo-responsive to NIT-1 cell lysate in vitro without inducing nonspecific immune suppression.

Given the repertoire of self-reactive, potentially pathogenic lymphocytes, therapeutic options to diminish autoimmune disease risk include deletion, reduced activation or increased regulation of self-reactive lymphocytes by means that mimic or promote physiological mechanisms of immunity [33]. Vaccination with self-antigen to promote self-antigen-specific tolerance, 'negative vaccination', may represent the most specific and potentially safest means of averting autoimmune disease. Although further studies are needed in order to deeply characterized the immune-mediated modulation achieved by this interesting technology, dendritic cell modulation represents an innovative and promising opportunity for diabetes treatment.

New genes

Meta-analysis of genome-wide association study data identifies additional type 1 diabetes risk loci

Cooper JD, Smyth DJ, Smiles AM, Plagnol V, Walker NM, Allen JE, Downes K, Barrett JC, Healy BC, Mychaleckyj JC, Warram JH, Todd JA

Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory, Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK

jason.cooper@cimr.cam.ac.uk

Nat Genet 2008;40:1399–1401

Methods: In this study a meta-analysis of data from 3 genome-wide association studies of T1D, testing 305,090 SNPs in 3,561 T1D cases and 4,646 controls of European ancestry was carried out by Cooper et al.

Results: The authors were able to demonstrate further support for 4q27 (IL2–IL21, $p = 1.9 \times 10^{-8}$) and, after genotyping an additional 6,225 cases, 6,946 controls and 2,828 families, obtained convincing evidence for 4 previously unknown and distinct risk loci in chromosome regions 6q15 (BACH2, $p = 4.7 \times 10^{-12}$), 10p15 (PRKCQ, $p = 3.7 \times 10^{-9}$), 15q24 (CTSH, $p = 3.2 \times 10^{-15}$) and 22q13 (C1QTNF6, $p = 2.0 \times 10^{-8}$).

Follow-up analysis of genome-wide association data identifies novel loci for type 1 diabetes

Grant SF, Qu HQ, Bradfield JP, Marchand L, Kim CE, Glessner JT, Grabs R, Taback SP, Frackelton EC, Eckert AW, Annaiah K, Lawson ML, Otieno FG, Santa E, Shaner JL, Smith RM, Skraban R, Imielinski M, Chiavacci RM, Grundmeier RW, Stanley CA, Kirsch SE, Waggott D, Paterson AD, Monos DS; DCCT/EDIC Research Group, Polychronakos C, Hakonarson H

Center for Applied Genomics, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, Penn., USA

hakonarson@chop.edu

Diabetes 2009;58:290–295

Background: Novel loci for T1D, a common multifactorial disease with a strong genetic component, have been shown in 2 recent genome-wide association studies. To fully utilize the genome-wide association data that Grant et al. had obtained by genotyping 563 T1D probands and 1,146 control subjects, as

well as 483 case subject-parent trios, using the Illumina HumanHap550 BeadChip, authors designed a full stage 2 study to capture other possible association signals.

Methods: From the previously designed datasets, Grant et al. selected 982 markers with $p < 0.05$ in both genome-wide association cohorts. Genotyping these in an independent set of 636 nuclear families with 974 affected offspring revealed 75 markers that also had $p < 0.05$ in this third cohort. Among these, 6 single nucleotide polymorphisms in 5 novel loci also had $p < 0.05$ in the Wellcome Trust Case-Control Consortium dataset and were further tested in 1,303 T1D probands from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) plus 1,673 control subjects.

Results: Two markers (rs9976767 and rs3757247) remained significant after adjusting for the number of tests in this last cohort; they reside in UBASH3A (OR 1.16; combined $p = 2.33 \times 10^{-8}$) and BACH2 (1.13; combined $p = 1.25 \times 10^{-6}$).

Conclusions: Additional loci associated with T1D have been revealed by evaluating a large number of statistical genome-wide association candidates in several independent cohorts. The two genes at these respective loci, UBASH3A and BACH2, are both biologically relevant to autoimmunity.

These 2 studies clearly show the ability of genome-wide analysis, especially if performed in a very large population-base database, to reveal new HLA or non-HLA risk loci for T1D. In particular, by forming a US case-control genome-wide association study from a smart meta-analysis of 3 genome-wide association studies, Cooper et al. were first able to provide further support for 4q27 (IL2-IL21) as an important T1D risk locus. More interestingly, they showed 4 previously unnoticed and different T1D risk loci (6q15, BACH2; 10p15, PRKCQ; 15q24, CTSN, and 22q13, C1QTNF6), increasing the total of T1D loci with convincing evidence from 10 [34] to 15 (including the HLA region). Similarly, by performing a full stage 2 study in which data from 4 independent studies were combined, Grant et al. were able to reveal 2 additional loci associated with T1D. In particular, they show 2 genes at these respective loci, UBASH3A (coding for 2 proteins STS1 and STS2 which are critical regulators of the signaling pathways that control T-cell activation) and BACH2 (encoding for a group of proteins of the small Maf family that seem to function as regulators of the antibody response), which result both biologically relevant to autoimmunity.

In addition to the detection of these new T1D risk loci, both studies were able to demonstrate the effectiveness of combining evidence from GWA studies to find disease loci, showing that genome-wide analysis studies can be successfully performed using case and control data from different studies. Therefore, the rapid diffusion of new methods able to carry out whole-genome association studies using a large number of single nucleotide polymorphisms will offer not only the possibility to perform a large genetic analysis, but will also represent publicly available resource data to further improve the power and effectiveness of analysis directed to evidence new T1D loci risk. In the future these new gene-detecting tools will allow a complete characterization of genetic risk factors related to T1D and will possibly allow a population-based analysis of genetic risk for T1D.

Genome-wide association scan for diabetic nephropathy susceptibility genes in type 1 diabetes mellitus

Pezzolesi MG, Poznik GD, Mychaleckyj JC, Paterson AD, Barati MT, Klein JB, Ng DP, Placha G, Canani LH, Bochenski J, Waggott D, Merchant ML, Krolewski B, Mirea L, Wanic K, Katavetin P, Kure M, Wolkow P, Dunn JS, Smiles A, Walker WH, Borchert AP, Bull SB; DCCT/EDIC Research Group, Doria A, Rogus JJ, Rich SS, Warram JH, Krolewski AS Research Division, Joslin Diabetes Center, and Department of Medicine, Harvard Medical School, Boston, Mass., USA andrzej.krolewski@joslin.harvard.edu
Diabetes 2009;58:1403–1410

Background: Despite extensive evidence for genetic susceptibility to diabetic nephropathy, limited success has been achieved in the identification of susceptibility genes and their variants. To search for diabetic nephropathy-related susceptibility genes, a genome-wide association scan was implemented on the Genetics of Kidneys in Diabetes collection.

Methods: Pezolesi et al. genotyped approximately 360,000 single nucleotide polymorphisms (SNPs) in 820 cases (284 with proteinuria and 536 with end-stage renal disease) and 885 controls with T1D. Confirmation of implicated SNPs was sought in 1,304 participants of the DCCT/EDIC study, a long-term, prospective investigation of the development of diabetes-associated complications.

Results: A total of 13 SNPs located in 4 genomic loci were associated with diabetic nephropathy with $p < 1 \times 10^{-5}$. The strongest association was at the *FRMD3* (FERM domain containing 3) locus ($OR = 1.45$, $p = 5.0 \times 10^{-7}$). A strong association was also identified at the *CARS* (cysteinyl-tRNA synthetase) locus ($OR = 1.36$, $p = 3.1 \times 10^{-6}$). Associations between both loci and time to onset of diabetic nephropathy were supported in the DCCT/EDIC study ($HR = 1.33$, $p = 0.02$ and $HR = 1.32$, $p = 0.01$, respectively). The expression of both *FRMD3* and *CARS* in the human kidney was demonstrated.

Conclusions: In this study Pezzolesi et al. were able to identify genetic associations for susceptibility to diabetic nephropathy at 2 novel candidate loci near the *FRMD3* and *CARS* genes. Their identification implicates previously unsuspected pathways in the pathogenesis of this important late complication of T1D.

In this study Pezzolesi et al. attempt to identify loci associated with the risk of diabetic nephropathy in patients with T1D included in the Genetics of Kidneys in Diabetes (GoKinD) collection (made up of 935 patients with T1D and 944 control subjects) [35]. In particular by performing a genome-wide scan of the entire population they were able to detect some significant associations with variants located within 4 distinct chromosomal regions whose biology, interestingly, remains to be elucidated. In detail, the regions detected implicate: *FRMD3*, encoding 4.10 protein (a structural protein with unknown function) and a member of the 4.1 family of proteins (a cytoskeletal proteins); *CARS*, encoding cysteinyl-tRNA synthetase (a regulators of intracellular amino acid concentrations and protein biosynthesis in both the cytoplasm and mitochondria); *CHN2/CPVL*, a carboxypeptidase that is highly expressed in the kidney; and an intergenic region on chromosome 13q, as novel genes/genetic regions involved in the pathogenesis of diabetic nephropathy which might also involve two major genes closest to the associated SNPs, *MYO16* (myosin heavy-chain Myr 8) and *IRS2* (insulin receptor substrate 2). Although the limitations related to this study (such as the small sample size, the study design which is weighted with case subjects with end stage renal diseases and especially the need to elucidate the mechanism underlying the revealed associations) these findings contribute to the understanding of genetic susceptibility of diabetic nephropathy in T1D. In fact, although mounting clinical and epidemiological evidence has shown a main role of genetic factors in diabetic nephropathy, up to now no genes have been unequivocally demonstrated. However, these findings are not surprising as, similar to other complex genetic disorders, no single major gene contributing to an increased risk of disease has emerged [36]. A complete understanding of the genetic predisposing factors will be helpful in defining patients with diabetes at increased risk of developing micro- and macrovascular complications. Therefore, a multicenter-wide population-based study needs to be designed to further characterize the pathogenic role of these and other candidate genes identified as genetic determinant of the diabetes-related vascular complications.

Two new hormones

Plasma osteoprotegerin levels predict cardiovascular and all-cause mortality and deterioration of kidney function in type 1 diabetic patients with nephropathy

Jorsal A, Tarnow L, Flyvbjerg A, Parving HH, Rossing P, Rasmussen LM

Steno Diabetes Center, Gentofte, Denmark

ajrs@steno.dk

Diabetologia 2008;51:2100–2107

Background: Osteoprotegerin, a bone-related peptide, is produced by vascular cells and is involved in the process of vascular calcification. The aim of this study was to investigate the predictive value of plasma levels of osteoprotegerin in relation to mortality, cardiovascular events and deterioration in kidney function in patients with T1D.

Methods: This prospective observational follow-up study included 397 patients with T1D and overt diabetic nephropathy (243 men; age [mean $\pm$ SD] 42.1 ± 10.6 years, duration of diabetes 28.3 ± 9.9 years, GFR 67 ± 28 ml min⁻¹ 1.73 m²) and a group of 176 patients with longstanding T1D and persistent normoalbuminuria (105 men; age 42.6 ± 9.7 years, duration of diabetes 27.6 ± 8.3 years).

Results: The median (range) follow-up period was 11.3 (0.0–12.9) years. Among patients with diabetic nephropathy, individuals with high osteoprotegerin levels (fourth quartile) had significantly higher all-cause mortality than patients with low levels (first quartile; covariate-adjusted hazard ratio [HR] 3.00 [1.24–7.27]). High osteoprotegerin levels also predicted cardiovascular mortality (covariate-adjusted HR 4.88 [1.57–15.14]). Furthermore, increased osteoprotegerin levels were associated with a significantly higher risk of progression to end-stage renal disease (covariate-adjusted HR 4.32 [1.45–12.87]) as well as a higher rate of decline in GFR.

Conclusions: High levels of osteoprotegerin predict all-cause and cardiovascular mortality in patients with diabetic nephropathy. Furthermore, high levels of osteoprotegerin predict deterioration of kidney function towards end-stage renal disease.

In this long prospective observational study, Jorsal et al. were able to reveal a novel independent risk marker for all-cause and cardiovascular mortality in a relatively large population of patients with T1D and overt nephropathy. In fact, during a 11.3-year follow-up period they showed that the higher the osteoprotegerin quartile range, the higher the risk of all-cause and cardiovascular mortality. They were also able to show that increased plasma osteoprotegerin concentrations predict deterioration of kidney function independent of potential influences of other well-known conventional cardiovascular and renal risk factors, including glomerular filtration rate. Although further larger and longer longitudinal studies are needed in order to confirm and validate these preliminary data, the identification of novel markers of cardiovascular diseases and deterioration of kidney function represents an important goal in patients with T1D. In fact, although microalbuminuria as well as HbA1c represent the best-available noninvasive and sensitive predictor of the risk of diabetic nephropathy as well as all-cause mortality and cardiovascular disease [37], some patients with microalbuminuria have advanced renal histopathological changes, and its full potential depends on longitudinal repeated measures [37]. Furthermore, although previous studies [20, 38] have demonstrated the importance of maintaining good glycemic control in the prevention of short- and long-term risk of complications, some patients develop chronic complications despite reasonable or even good metabolic control. Therefore, the identification of novel and highly sensitive and specific markers of risk of vascular complications in patients with T1D are needed.

Liraglutide, a long-acting human glucagon-like peptide 1 analog, improves glucose homeostasis in marginal mass islet transplantation in mice

Merani S, Truong W, Emamaullee JA, Toso C, Knudsen LB, Shapiro AM
Surgical Medical Research Institute, University of Alberta, Edmonton, Alta., Canada
shapiro@islet.ca
Endocrinology 2008;149:4322–4328

Background: The current scarcity of high-quality deceased pancreas donors represents a relevant factor able to minimize the widespread application of islet transplantation for treatment of labile T1D. Opportunities for the improvement of current techniques include optimization of islet isolation and purification, use of culture with pharmacological insulintropic agents, strategies to reduce graft rejection and inflammation, and the search for alternative insulin-producing tissue.

Methods: In this study Merani et al. reported findings on the efficacy of the long-acting human glucagon-like peptide 1 analog, liraglutide, in a mouse model of marginal mass islet transplantation. In streptozotocin-induced diabetic BALB/c mice liraglutide was administered (200 µg/kg s.c. twice daily) after a marginal mass syngeneic islet transplant.

Results: In liraglutide-treated animals the time-to-normoglycemia was significantly shorter (median 1 vs. 7 days; $p = 0.0003$), even in recipients receiving sirolimus (median 1 vs. 72.5 days; $p < 0.0001$). Furthermore, improved glucose tolerance, as assessed by an intraperitoneal glucose tolerance test, was shown in liraglutide-treated animals. Liraglutide discontinuation on postoperative day 90 resulted in diminished glucose tolerance during the intraperitoneal glucose tolerance test, whereas a late-start liraglutide therapy 90 days after transplant resulted in no improvement. These findings suggest that liraglutide therapy mediates early and late insulintropic effects. In accordance with this hypothesis, insulin/terminal deoxynucleotidyl transferase-mediated deoxyuridine 5-triphosphate nick end-labeling fluorescence microscopy showed reduced transplanted β -cell apoptosis in liraglutide-treated recipients 48 h after transplant. In addition, liraglutide resulted in improved glucose-dependent insulin secretion.

Conclusions: In conclusion, this study showed that liraglutide has a beneficial impact on the engraftment and function of syngeneic islet transplants in mice, when administered continuously starting on the day of transplant.

In this study Merani et al. attempted to evaluate whether liraglutide, a glucagon-like peptide 1 (GLP-1) analog, might represent a new clinically applicable therapeutic opportunity able to prolong and improved transplanted islet in patients with T1D. In particular, after transplanting diabetic BALB/c animals with a marginal mass of 250 syngeneic islets, animals were randomized to 4 treatment groups: vehicle; liraglutide; sirolimus (an immunosuppressive drug); or sirolimus plus liraglutide. Thus, they showed that liraglutide administration reduced the time required to achieve normoglycemia (time to engraftment) in transplanted animals as well as provided better long-term glycemic control and improved glucose homeostasis in the marginal mass islet transplant model without the need for exogenous insulin. Furthermore, they also demonstrated that these characteristics of liraglutide are present even when sirolimus is administered concurrently. In addition Merani et al. have offered relevant information on the time of liraglutide administration as well on the mechanism of liraglutide action. In fact, they clarified that liraglutide must be administered both immediately after islet transplantation and continuously thereafter to maximize its beneficial effects. In addition, they also showed that liraglutide administration results in both reduced β -cell apoptosis and improved glucose-dependent insulin secretion. Although further investigation on liraglutide in the field of islet transplantation are needed, the effect of liraglutide on β -cell activity is promising. In fact, although islet transplantation is an emerging therapeutic option for the treatment of select labile T1D, several barriers prevent its broad application, even within a selected group of patients [39]. In particular, the frequently documented insulin requirement even after islet transplantation and the insurgence of islet allograft rejection minimize its application [39]. Because of the liraglutide effects on reduced apoptosis in transplanted β cells and on improved glucose metabolism, it might be speculated that a complete characterization of the GLP-1 analog-related actions would offer relevant help by delaying islet allo-rejection and by inducing a complete insulin independency.

Review

Clinical review 2: The 'metabolic memory': is more than just tight glucose control necessary to prevent diabetic complications?

Ceriello A, Ihnat MA, Thorpe JE
Warwick Medical School, University of Warwick, Coventry, UK
antonio.ceriello@warwick.ac.uk
J Clin Endocrinol Metab 2009;94:410–415

Background: The concept of a 'metabolic memory', that is of diabetic vascular stresses persisting after glucose normalization, has been supported both in the laboratory and in the clinic and in T1D as well as in T2D.

Methods: Using PubMed sources, Ceriello et al. searched for publications on diabetic micro- and macro-vascular complications using terms such as persistence, prolongation, sustained, and 'memory', and focused on the mechanistic basis behind this metabolic memory.

Results: They found that as early as the mid-1980s this memory phenomenon was described in diabetic animals and isolated cells exposed to high glucose followed by normalized glucose and then, beginning around 2002, in results from large clinical trials such as the DCCT-EDIC and the United Kingdom Prospective Diabetes Study. Furthermore, mechanisms for propagating this memory appear focused on the non-enzymatic glycation of cellular proteins and lipids and on an excess of cellular reactive oxygen and nitrogen species, in particular originating at the level of glycated mitochondrial proteins and perhaps acting in concert with one another to maintain stress signaling independent of glucose levels.

Conclusions: The relevant role of this metabolic memory suggests either the need for early aggressive treatment aiming to 'normalize' metabolic control or the addition of agents which reduce cellular reactive species and glycation in order to minimize long-term diabetic complications.

During the last 50 years several in vitro and in vivo (both in animals and humans), studies have clearly demonstrated that hyperglycemia represents the major determinant of the development and progression of diabetes related micro- and macrovascular complications [21, 38]. Further studies have unequivocally demonstrated that the effects of hyperglycemia exposure persist even after a complete metabolic normalization introducing the concept of the 'metabolic memory'. By reviewing published data using the PubMed source, Ceriello et al. attempt to give a complete view of the in vitro and in vivo evidence of the existing 'metabolic memory' in patients with diabetes. In particular, by unifying different evidence, they clearly showed that hyperglycemia has long-lasting deleterious effects both in T1D and T2D and that glycemic control, if not started at a very early stage of the disease, is not sufficient to completely reduce the risk of vascular complications. In addition, Ceriello et al. described the main mechanisms implicated in 'metabolic memory'. This evidence raises many questions regarding the therapeutic management of diabetes. In particular, the existence of the metabolic memory suggests that very early aggressive treatment of hyperglycemia is mandatory. As it is documented that despite the awareness of the importance of maintaining good glycemic control, treatment of diabetes is inadequate, especially in childhood [40]. Therefore, Ceriello et al. prospect a future strategy consisting not only in an early aggressive treatment of hyperglycemia, but with the simultaneous use of compounds active on advanced glycated end-product formation, together with compounds capable of specifically targeting mitochondrial reactive species which have been shown to play the main role in the instauration of a metabolic memory. This strategy might have the potential to reduce the deleterious effects of metabolic memory and hyperglycemia on diabetic complications.

Food for thought

Shared and distinct genetic variants in type 1 diabetes and celiac disease

Smyth DJ, Plagnol V, Walker NM, Cooper JD, Downes K, Yang JH, Howson JM, Stevens H, McManus R, Wijmenga C, Heap GA, Dubois PC, Clayton DG, Hunt KA, van Heel DA, Todd JA

Juvenile Diabetes Research Foundation-Wellcome Trust Diabetes and Inflammation Laboratory, Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK

john.todd@cmr.cam.ac.uk

N Engl J Med 2008;359:2767–2777

Background: Two inflammatory disorders, T1D and celiac disease, are frequently associated in populations, suggesting a common genetic origin. Since the HLA class II genes on chromosome 6p21 are associated in both diseases, Smyth et al. tested whether non-HLA loci are shared.

Methods: They evaluated the association between T1D and 8 loci related to the risk of celiac disease by genotyping and statistical analyses of DNA samples from 8,064 patients with T1D, 9,339 control subjects, and 2,828 families providing 3,064 parent-child trios (consisting of an affected child and both biologic parents). They also investigated 18 loci associated with T1D in 2,560 patients with celiac disease and 9,339 control subjects.

Results: Three celiac disease loci – RGS1 on chromosome 1q31, IL18RAP on chromosome 2q12, and TAGAP on chromosome 6q25 – were associated with T1D ($p < 1.00 \times 10^{-4}$). The 32-bp insertion-deletion variant on chromosome 3p21 was newly identified as a T1D locus ($p = 1.81 \times 10^{-8}$) and was also associated with celiac disease, along with PTPN2 on chromosome 18p11 and CTLA4 on chromosome 2q33, bringing the total number of loci with evidence of a shared association to 7, including SH2B3 on chromosome 12q24. The effects of the IL18RAP and TAGAP alleles confer protection in T1D and susceptibility in celiac disease. INS on chromosome 11p15, IL2RA on chromosome 10p15, and PTPN22 on chromosome 1p13 in T1D, and IL12A on 3q25 and LPP on 3q28 in celiac disease were shown to be loci with distinct effects in the two diseases.

Conclusions: A genetic susceptibility to both T1D and celiac disease shares common alleles. Therefore, common biologic mechanisms, such as autoimmunity-related tissue damage and intolerance to dietary antigens, may be etiologic features of both diseases.

By genotyping and statistically analyzing data from a large population, Smyth et al. explored the existence of non HLA-related loci associated with T1D and celiac disease and shared by the 2 diseases. In particular, according to the results obtained by previously published genome-wide association studies, they genotyped single-nucleotide polymorphisms (SNPs) from 8 celiac disease loci and from 15 T1D loci as well as from 3 other major genes (IL7R, CD226, and the 32-bp insertion–deletion variant in CCR5).

According to previous studies, Smyth et al. provided further evidence that 21 non-HLA loci are associated with T1D and 11 non-HLA loci are associated with celiac disease. In addition, in patients with T1D statistical analysis showed that 3 of the non-HLA regions associated with celiac diseases (in particular RGS1 on chromosome 1q31, IL18RAP on chromosome 2q12, and TAGAP on chromosome 6q25) proved to be strong evidence for the association with diabetes. On the other hand, in patients with celiac disease, Smyth et al. demonstrated in a logistic regression analysis that 7 T1D loci (RGS1, TAGAP, IL18RAP, CTLA4 on chromosome 2q33, CCR5 on chromosome 3p21, SH2B3 on chromosome 12q24, and PTPN2 on chromosome 18p119) are convincing evidence of an association with the disease. In addition, RGS1, CTLA4, SH2B3, and PTPN2 showed the same direction of association in the 2 diseases, constituting evidence for shared causal variants. In contrast, the minor alleles of the SNPs rs917997 (IL18RAP) and rs1738074 (TAGAP) were negatively associated with T1D, whereas these minor alleles were positively associated with celiac disease.

In conclusion, 7 chromosome regions, 3 celiac disease loci, associated with T1D (RGS1, IL18RAP, and TAGAP), and 2 T1D loci, associated with celiac disease (CCR5 and PTPN2), are shared between the 2 diseases, giving clear evidence of a common genetic susceptibility pattern. As these loci are directly involved in different cellular or immunological functions, the complete characterization of the genetic-dependent determinant shared by both diseases will help to offer further pieces of the complex puzzle describing these multifaceted diseases. In addition, full investigation of any possible association between the different genetic risk determinants and the natural history of diabetes or celiac onset in both these autoimmune diseases will offer further help for evaluating new approaches to preventing or retarding their onset.

Iodine and tri-iodo-thyronine reduce the incidence of type 1 diabetes mellitus in the autoimmune prone BB rats

Hartoft-Nielsen ML, Rasmussen AK, Bock T, Feldt-Rasmussen U, Kaas A, Buschard K

Department of Endocrinology, Rigshospitalet, University Hospital of Copenhagen, Copenhagen, Denmark

ma-lars@dadlnet.dk

Autoimmunity 2009;42:131–138

Background: Thyroid hormones modulate the immune system and metabolism, influence insulin secretion, and cause decreased glucose tolerance. Although it has been described that thyroid hormones are able to change the incidence of spontaneous autoimmune thyroiditis in Bio-Breeding/Worcester (BB) rats, whether these hormones do or do not affect the development of T1D is still unknown. The aim of this study was to investigate the influence of changes in thyroid function during postnatal development on the prevalence of T1D in BB rats and the influence of T₃ on the β -cell mass in non-diabetic Wistar rats.

Methods: BB rats were treated with sodium iodine (NaI) or thyroid-stimulating hormone (TSH) neonatally, or with tri-iodo-thyronine (T₃) during adolescence. The incidence of T1D and the degree of insulinitis were evaluated at the age of 19 weeks. By unbiased stereological methods, the influence of T₃ treatment on the β -cell mass was evaluated in Wistar rats.

Results: The incidence of T1D in control BB rats was 68% at the age of 19 weeks. NaI and T₃ reduced the incidence, whereas TSH had no effect. In Wistar rats T₃ treatment increased the β -cell mass per body-weight.

Conclusions: The modulation of thyroid function during postnatal development may affect the precipitation of T1D in genetically susceptible individuals.

In this study Hartoft-Nielsen et al. were able, for the first time, to investigate the influence of thyroid hormones on the incidence of T1D in rats. In particular, by performing analysis in the BB rat (a well-established animal model with a spontaneous incidence of about 50% for T1D and autoimmune thyroiditis), they showed that treatment with thyroid hormone and iodine significantly reduces the

incidence of T1D in BB rats. In fact, in male rats, iodine given immediately postpartum and thereafter daily for the first 6 days reduced the incidence of T1D; in contrast, no effects on the T1D development were documented after TSH administration (given immediately postpartum and daily for the first 3 days during the first 3 weeks). Similar to iodine treatment, the authors reported that T₃ given during adolescence reduced the incidence of T1D but only significantly in female rats, with a protective effect of iodine which seems to be stronger than the protective effect of T₃. Although these results appear quite interesting, the mechanisms of action for the reduced incidence of T1D after the iodine and T₃ treatment are not known and several hypothesis might be postulated. In fact, for both treatments the effect could be a direct influence on the β cells or a more indirect effect on the immune system. For example, treatment could act by influencing the functional state of β cells and therefore the important balance between apoptosis, replication and neogenesis. This might be caused by a reduced endogenous insulin secretion and reduced amount of antigens on the β -cell surface [41]. Furthermore, resting β cells are less vulnerable to a destructive effect of proinflammatory cytokines. In addition, as the treatment is given earlier, different mechanisms might be moved on for iodine treatment. In particular, as documented in β cells treated neonatally with stimulatory drugs, iodine might cause an early maturation of the β cells, resulting in a stronger recognition of the β cells as 'self' by the immune system. However, up to now no exact mechanisms have been defined. Finally, because of the influence of T₃ on glucose metabolism, and of the influence of iodine and T₃ on metabolism in general [42], the complete characterization of the underlying mechanisms represents an important tool to be defined in order to offer new prospective views involved in the development of T1D and autoimmune thyroiditis.

References

- Shoda LK, Young DL, Ramanujan S, Whiting CC, Atkinson MA, Bluestone JA, et al: A comprehensive review of interventions in the NOD mouse and implications for translation. *Immunity* 2005;23:115–126.
- Lee DD, Grossman E, Chong AS: Cellular therapies for type 1 diabetes. *Horm Metab Res* 2008;40:147–154.
- Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, Hale G, et al: Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med* 2005;352:2598–2608.
- Hochedlinger K, Jaenisch R: Nuclear reprogramming and pluripotency. *Nature* 2006;441:1061–1067.
- Orkin SH, Zon LI: Hematopoiesis: an evolving paradigm for stem cell biology. *Cell* 2008;132:631–644.
- Pearl EJ, Horb ME: Promoting ectopic pancreatic fates: pancreas development and future diabetes therapies. *Clin Genet* 2008;74:316–324.
- Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, et al: Effects of oral insulin in relatives of patients with type 1 diabetes: the Diabetes Prevention Trial – Type 1. *Diabetes Care* 2005;28:1068–1076.
- Diabetes Prevention Trial–Type 1 Diabetes Study Group: Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N Engl J Med* 2002;346:1685–1691.
- Kobayashi T, Maruyama T, Shimada A, Kasuga A, Kanatsuka A, Takei I, et al: Insulin intervention to preserve beta cells in slowly progressive insulin-dependent (type 1) diabetes mellitus. *Ann NY Acad Sci* 2002;958:117–130.
- Toma A, Laika T, Haddouk S, Luce S, Briand JP, Camoin L, et al: Recognition of human proinsulin leader sequence by class I-restricted T-cells in HLA-A*0201 transgenic mice and in human type 1 diabetes. *Diabetes* 2009;58:394–402.
- Keller RJ, Eisenbarth GS, Jackson RA: Insulin prophylaxis in individuals at high risk of type 1 diabetes. *Lancet* 1993;341:927–928.
- Montanya E, Fernandez-Castaner M, Soler J: Improved metabolic control preserved beta-cell function two years after diagnosis of insulin-dependent diabetes mellitus. *Diabetes Metab* 1997;23:314–319.
- Effect of intensive therapy on residual beta-cell function in patients with type 1 diabetes in the diabetes control and complications trial. A randomized, controlled trial. The Diabetes Control and Complications Trial Research Group. *Ann Intern Med* 1998;128:517–523.
- Vang T, Miletic AV, Arimura Y, Tautz L, Rickert RC, Mustelin T: Protein tyrosine phosphatases in autoimmunity. *Annu Rev Immunol* 2008;26:29–55.
- Magro F, Costa C: Long-standing remission of Crohn's disease under imatinib therapy in a patient with Crohn's disease. *Inflamm Bowel Dis* 2006;12:1087–1089.
- Hagerkvist R, Sandler S, Mokhtari D, Welsh N: Amelioration of diabetes by imatinib mesylate (Gleevec): role of beta-cell NF-kappaB activation and anti-apoptotic preconditioning. *FASEB J* 2007;21:618–628.
- Liu EY, Wactawski-Wende J, Donahue RP, Dmochowski J, Hovey KM, Quattrin T: Does low bone mineral density start in post-teenage years in women with type 1 diabetes? *Diabetes Care* 2003;26:2365–2369.
- Rakel A, Sheehy O, Rahme E, LeLorier J: Osteoporosis among patients with type 1 and type 2 diabetes. *Diabetes Metab* 2008;34:193–205.
- Forsen L, Meyer HE, Midthjell K, Edna TH: Diabetes mellitus and the incidence of hip fracture: results from the Nord-Trøndelag Health Survey. *Diabetologia* 1999;42:920–925.
- The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med* 1993;329:977–986.
- The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. *Diabetes* 1995;44:968–983.
- Prince CT, Becker DJ, Costacou T, Miller RG, Orchard TJ: Changes in glycaemic control and risk of coronary artery disease in type 1 diabetes mellitus: findings from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC). *Diabetologia* 2007;50:2280–2288.

23. Monnier L, Lapinski H, Colette C: Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). *Diabetes Care* 2003;26:881–885.
24. Coutinho M, Gerstein HC, Wang Y, Yusuf S: The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. *Diabetes Care* 1999;22:233–240.
25. Roach P: New insulin analogues and routes of delivery: pharmacodynamic and clinical considerations. *Clin Pharmacokinet* 2008;47:595–610.
26. Gough SC: A review of human and analogue insulin trials. *Diabetes Res Clin Pract* 2007;77:1–15.
27. Steffes MW, Sibley S, Jackson M, Thomas W: Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care* 2003;26:832–836.
28. Nakanishi K, Watanabe C: Rate of beta-cell destruction in type 1 diabetes influences the development of diabetic retinopathy: protective effect of residual beta-cell function for more than 10 years. *J Clin Endocrinol Metab* 2008;93:4759–4766.
29. Cryer PE: Hypoglycemia unawareness in IDDM. *Diabetes Care* 1993;16(suppl 3):40–47.
30. Schleis T: Realities of the fluoxetine-to-sertraline switch. *Am J Health Syst Pharm* 1995;52:423; author reply 4, 8.
31. Carvalho F, Barros D, Silva J, Rezende E, Soares M, Fregoneze J, et al: Hyperglycemia induced by acute central fluoxetine administration: role of the central CRH system and 5-HT3 receptors. *Neuropeptides* 2004;38:98–105.
32. McCurry KR, Colvin BL, Zahorchak AF, Thomson AW: Regulatory dendritic cell therapy in organ transplantation. *Transpl Int* 2006;19:525–538.
33. Harrison LC: Vaccination against self to prevent autoimmune disease: the type 1 diabetes model. *Immunol Cell Biol* 2008;86:139–145.
34. Hakonarson H, Qu HQ, Bradfield JP, Marchand L, Kim CE, Glessner JT, et al: A novel susceptibility locus for type 1 diabetes on Chr12q13 identified by a genome-wide association study. *Diabetes* 2008;57:1143–1146.
35. Mueller PW, Rogus JJ, Cleary PA, Zhao Y, Smiles AM, Steffes MW, et al: Genetics of Kidneys in Diabetes (GoKinD) study: a genetics collection available for identifying genetic susceptibility factors for diabetic nephropathy in type 1 diabetes. *J Am Soc Nephrol* 2006;17:1782–1790.
36. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al: A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 2007;316:1341–1345.
37. Mogensen CE, Poulsen PL: Microalbuminuria, glycemic control, and blood pressure predicting outcome in diabetes type 1 and type 2. *Kidney Int Suppl* 2004;92:S40–S41.
38. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. *JAMA* 2002;287:2563–2569.
39. Leitão CB, Cure P, Tharavani T, Baidal DA, Alejandro R: Current challenges in islet transplantation. *Curr Diab Rep* 2008;8:324–331.
40. Danne T, Mortensen HB, Hougaard P, Lynggaard H, Aanstoet HJ, Chiarelli F, et al: Persistent differences among centers over 3 years in glycemic control and hypoglycemia in a study of 3,805 children and adolescents with type 1 diabetes from the Hvidovre Study Group. *Diabetes Care* 2001;24:1342–1347.
41. Atkinson MA, Maclaren NK, Luchetta R: Insulinitis and diabetes in NOD mice reduced by prophylactic insulin therapy. *Diabetes* 1990;39:933–937.
42. Chidakel A, Mentuccia D, Celi FS: Peripheral metabolism of thyroid hormone and glucose homeostasis. *Thyroid* 2005;15:899–903.