

Autoimmunity special issue

Immunomodulation with human recombinant autoantigens

Åke Lernmark¹ and Carl-David Agardh²

¹The University of Washington, Department of Medicine, Seattle, WA 981905, USA

²Lund University, Department of Clinical Sciences, Malmö, University hospital MAS, SE-20502 Malmö, Sweden

The loss of β cells in type 1 diabetes is the consequence of a T cell-dependent autoimmune attack. Autoantibodies against GAD65 (Mr 65.000 isoform of glutamic acid decarboxylase), IA-2 (insulinoma-associated protein IA-2) or insulin, alone or in combination, predict disease. Preclinical studies in spontaneously diabetic rodents suggest that immunomodulation with autoantigens might alter the course of autoimmune diabetes. Oral insulin reduces the development of diabetes in risk subjects with high insulin autoantibody levels. Giving alum-formulated GAD65 to patients classified with latent autoimmune diabetes of the adult (LADA) is safe and suggests possible immunomodulating effects of GAD65. Future immunomodulation trials might better ascertain subjects based on HLA genetic risk factors, the level of insulin that is still produced or by combining autoantigens with, for example, anti-CD3 antibodies, to induce antigen-specific tolerance and thereby a long-lasting protection for β cells.

Human type 1 diabetes and markers of islet autoimmunity

The clinical onset of type 1 diabetes is associated with a major loss of pancreatic β cells. The loss is age-dependent and, whereas children have little β -cell function left at the time of diagnosis, adults might be diagnosed with hyperglycemia, despite the residual insulin-production being well within the normal range [1,2]. The variability in the age at onset is thought to be dependent on other factors, such as insulin sensitivity [3]. Residual β -cell function is determined by plasma c-peptide measurements but it is still unclear to what extent plasma c-peptide levels reflect the β -cell mass accurately. At the time of clinical onset, the β -cell volume has been reduced by 80–90% and it is well documented that some, but not all, islets are infiltrated by mononuclear cells, including macrophages, T and B cells [4,5]. Because patients usually survive the onset of diabetes, there are limited quantitative studies of the pancreas on the day of diagnosis and little data on islet cell autoantibody-positive subjects [5]. The disease can be transmitted, although not always [6], by bone marrow transplantation [7] and the development of the disease in a patient with X-linked

agammaglobulinemia underscores the importance of T cells [8]. It is therefore not fully clarified what (and when) the contributions of macrophages and dendritic cells (DCs), as well as T and B cells, are to the insulinitis process during the long prodrome of islet autoimmunity.

T- and B-cell predictive markers in type 1 diabetes

The compelling evidence in numerous animal, and fewer human, investigations identifies a major role for T cells in the pathogenesis of type 1 diabetes. Cytotoxic CD8⁺ T cells, restricted by HLA-A1 and -B8, recognize proinsulin [9] or preproIAPP [10]. However, these and similar tests with other islet autoantigens, such as IA-2 (insulinoma-associated protein IA-2) [11] or glucose-6-phosphatase [12], need to be replicated and subjected to standardization [13] to establish their clinical effectiveness.

The same is true for the many studies of CD4⁺ T cells in type 1 diabetes. CD4⁺ T cells were demonstrated recently in fresh blood from patients with type 1 diabetes by soluble tetramer preparations [14]. New onset type 1 diabetes patients and a few subjects at risk had CD4⁺ T cells restricted by HLA-DR B1*0401 or B1*0404 and the GAD65 (Mr 65.000 isoform of glutamic acid decarboxylase) epitope 555–567, whereas such cells were not detected in controls [15]. However, by the time subjects develop type 1 diabetes, they have T-cell responses to numerous islet proteins, whereas T cells from normal controls respond to a limited number of islet proteins [16]. It is sad to conclude that, at present, there is not a standardized T-cell test for type 1 diabetes risk, let alone type 1 diabetes at the time of clinical diagnosis [17]. The ideal T-cell assay would be one that has a positive predictive value that exceeds that of current standardized islet autoantibody tests [18].

Why has it not been possible to correlate a loss of β cells with peripheral blood T-cell tests? Apart from the lack of a standardized T-cell test, another possible explanation is that such T cells do not appear within the circulation but are confined to the islets themselves and/of the lymph nodes that drain the pancreas [19].

Islet autoantibody markers in diabetes

In contrast to T-cell tests, analyses of autoantibodies are either fully standardized (GADA, IA-2A) (Table 1) or are in the process of being standardized [insulin autoantibodies

Corresponding author: Lernmark, Å (ake@u.washington.edu).

Available online 8 September 2005

Table 1. Major autoantigens and autoantibodies in type 1 diabetes

Autoantigen	Autoantibody	HLA-association with autoantibody	Diagnostic sensitivity ^a	Diagnostic specificity
Insulin	IAA	DR4-DQA1*0301-B1*0302	30–60%	1%
GAD65	GAD65Ab	DR3-DQA1*0501-B1*0201	60–80%	1%
IA-2	IA-2Ab	DR4-DQA1*0301-B1*0302	30–60%	0.5%

^aThe diagnostic sensitivity is markedly age-dependent: IAA and IA-2A are found in young subjects; GAD65Ab is less affected by age.

(IAA) [18]. Furthermore, the use of the tests for GADA, IA-2A and IAA has shown that these autoantibodies, alone or in combination, predict disease [20,21]. It has also been suggested that autoantibody isotypes and subtypes reflect T-cell activities and, because the autoantibody assays are robust, it is often argued that autoantibody tests are surrogate measures of T-cell activities. The development of coupled *in vitro* transcription–translation using GAD65 cDNA [22], which maintains crucial conformational epitopes lost in ELISA-type assays, resulted in a rapid standardization of both GAD65 and IA-2 autoantibodies in international serum exchange workshops [23], a WHO reference serum [24] and the Diabetes Antibody Standardization Program (DASP) [18]. GAD65 antibody (Ab) and IA-2Ab have reached high levels of inter-laboratory concordance, whereas current IAA assays are still disparate and in need of a reference serum [18].

Islet autoantibodies as inclusion criteria in intervention trials

GAD65Ab, IA-2A and IAA (Table 1) demonstrate not only age-dependent high diagnostic sensitivity and specificity for type 1 diabetes [25,26] but also significant predictive value among first degree relatives to patients with type 1 diabetes as well as within the general population (reviewed in Refs [27–29]). In adult diabetic patients, who do not require insulin therapy for at least 6 months after diagnosis, the GAD65Ab identifies the so-called latent autoimmune diabetes in adults (LADA) (reviewed in Ref. [2]). Taking numerous studies together, analyses of standardized islet autoantibodies fulfill sufficient criteria for type 1 diabetes risk in longitudinal observational clinical trials, such as BABYDIAB [30], DIASY [31], PANDA [32] and DIPP [33], as well as TEDDY (www.teddystudy.org/) and others. Islet cell autoantibody positivity is also an inclusion criterion in large intervention studies using either parenteral insulin in the Diabetes Prevention Trial-1 (DPT-1) [34] or nicotinamide in the European Nicotinamide Diabetes Intervention Trial (ENDIT) [35]. Although neither study showed any effect of the treatment, both studies showed the reproducibility of islet autoantibody prediction for type 1 diabetes. TrialNet, a network of clinical centers working in cooperation with screening sites throughout the United States, Canada, Finland, UK, Italy, Germany, Australia and New Zealand, also uses islet cell autoantibody tests to identify subjects for immune intervention trials (www.bsc.gwu.edu/trialnet/) (Box 1). In TrialNet, new onset type 1 diabetes patients are currently recruited to a study with Mycophenolate mofetil (MMF/CellCept[®]) and Daclizumab (DZB/Zenapax[®]), alone, replaced with placebo or in

combination. Other studies are in the planning stages or are soon to begin, by other organizations, such as the Immune Tolerance Network (www.immunetolerance.org/) (Box 1).

Box 1. Immunomodulation trials in type 1 diabetes

Immunomodulation is approached in two groups of subjects. The first group, subjects at risk, represents individuals who have a father, mother or sibling with type 1 diabetes (first degree relatives). These subjects are randomized to treatment if they are positive for autoantibodies to GAD65, IA-2 or insulin, alone or in combination. The second group, newly diagnosed type 1 diabetes patients, are typically randomized to treatment within months following the diagnosis of diabetes and classification of type 1 diabetes based on clinical criteria.

Subjects at risk (islet autoantibody-positive first degree relatives)

- Casein hydrolysate (Nutramigen[™])
Trial to reduce insulin-dependent diabetes mellitus (IDDM) in the genetically at risk (TRIGR): newborns with a first degree relative with diabetes are randomized to the test or control formula without or with cow's milk protein [ongoing, supported by the National Institutes of Health (NIH)].
- Docosahexaenoic acid (DHA)
Nutritional intervention to prevent (NIP) diabetes study by TrialNet in pregnant mothers with a first degree relative with diabetes (begins in 2005, supported by TrialNet).
- Oral insulin
Delay in subjects with high IAA levels (a new study will be carried out by TrialNet).
- Exenatide
Beta cell preservation pilot study (supported by TrialNet).
- Anti-CD3
TrialNet study in the planning phase.

Newly diagnosed type 1 diabetes patients

- Mycophenolate mofetil (MMF/CellCept[®]) and Daclizumab (DZB/Zenapax[®]) (POPPII-1 is an ongoing TrialNet study)
- Antithymocyte globulin (ATG)
Thymoglobulin[®] treatment [supported by the Immune Tolerance Network (ITN), NIH, protocol in development].
- Anti-CD3
New trial proposed as a placebo-controlled, randomized, double-blind study (supported by ITN, TrialNet and NIH).
- Anti-CD3 and exenatide
Combination therapy planned by TrialNet.
- Anti-CD20
Rituxan[®] (Rituximab) is in the planning phase by TrialNet.
- Insulin B-chain in IFA
IBC-VS01 is tested in a Phase I clinical trial, supported by the ITN.
- HSP peptide
DiaPep 277 is in Phase II and Phase III clinical trial in patients with LADA, sponsored by DeveloGen, Göttingen, Germany.
- Alum-formulated GAD65
Diamyd[®] is in Phase IIb clinical trials in both new onset type 1 diabetes and LADA patients, sponsored by Diamyd Medical AB, Stockholm, Sweden.
- Insulin altered peptide ligand
NBI-6024[®] is in Phase IIb clinical trials sponsored by Neurocrine Biosciences San Diego CA.

Immune intervention trials in type 1 diabetes

The first report of immune intervention in type 1 diabetes was by David Pyke, who treated two patients with intravenous cortisone in 1976. The treatment was carried out within two years of it being demonstrated in 1974 that type 1 diabetes was associated with both HLA [36] and islet cell autoantibodies [37]. This initial attempt was followed by numerous, mostly open uncontrolled smaller clinical trials with a host of immunosuppressive reagents (reviewed in Refs [38,39]) or immunomodulation, including plasmapheresis [40,41]. Two independent placebo-controlled trials with cyclosporine showed a transient effect on the preservation of c-peptide production in new onset type 1 diabetes patients [42–44]; however the treatment of new onset type 1 diabetes patients was abandoned because of transient effects and of side effects that sometimes [45], but not always [46], affected the kidneys. The results from the cyclosporine trial were, however, encouraging. It was encouraging because it was the first time that an immunosuppressive agent preserved endogenous β -cell function in a controlled trial, albeit in a transient manner.

Subsequent placebo-controlled immune intervention trials were directed either at islet cell autoantibody-positive subjects at risk of type 1 diabetes or in newly diagnosed type 1 diabetes patients. In subjects at risk, parenteral insulin was administered in the DPT-1 trial [34] and nicotinamide in the ENDIT study [35]. In new onset patients, a heat-shock protein (HSP) peptide (DiaPep277) [47] or anti-CD3 monoclonal antibodies [48,49] were tested. All of these, and some other approaches, have been tested in the spontaneously diabetic non-obese diabetic (NOD) mouse and some of them also in the BB rat, which has been reviewed in detail in the accompanying paper of Serreze and Chen. In fact, the use of the NOD mouse in formal preclinical trials has been questioned because the progression towards diabetes appears too easy to influence [50,51]. As pointed out by Serreze and Chen, the observations in the NOD mouse that diabetes is delayed or prevented by parenteral insulin and nicotinamide has not been replicated in the human clinical trials. Similarly, since the first studies showing that NOD mouse diabetes was prevented by GAD65 [52,53], a wide variety of approaches to study, not only GAD65, but also insulin and insulin peptides in the NOD mouse have shown different effects on the disease process.

Although most studies use onset of diabetes as the endpoint, treatment with CD3 monoclonal antibodies indeed reversed the onset of the disease [54]. This is an important distinction. Otherwise, it would seem that neither the NOD mouse nor the BB rat are likely to be suitable for the design of human immune intervention clinical trials because these animals are rodents and their immune response biology is separated from humans by several million years. This does not take away the importance of these animals in terms of studies of mechanisms using genetic and molecular approaches that cannot be used in humans.

Human immune intervention trials need to be designed based on what can be measured most reliably in humans, that is, residual c-peptide as a measure of β -cell function

and GAD65Ab and IA-2Ab, as well as, to a certain extent, IAA, as a measure of islet autoimmunity. An important question is, therefore, whether immunomodulation regimens are able to alter the levels of these autoantibodies in subjects at risk or in newly diagnosed diabetes patients. So far, to our knowledge, none of the human clinical trials showed effects that reduced islet cell autoantibody levels. This would seem to warrant immunomodulation studies with GAD65, IA-2 and insulin.

Antigen-specific immunomodulation in autoimmune diabetes

There are limited examples of immunotherapies in humans that use specific antigens, other than antigens that occur on cancer cells [55,56]. Clinical regressions of human solid tumors have occurred with some cancer vaccines, however, the rate of effective responses remains low. Immune tolerance induction with factor VIII is being used with better success in the treatment of hemophilia [57]. The understanding of immunological tolerance and the possibilities of manipulating this phenomenon are vast in the mouse but translation to humans has been limited. Although parenteral or oral insulin treatment of subjects at risk of type 1 diabetes does not protect from disease [34,58], a subanalysis shows that clinical onset is reduced in a subgroup of high risk subjects who had high levels of IAA when they entered into the study [58].

The Immune Tolerance Network is, in addition, carrying out a Phase I clinical trial with the b-chain of insulin administered in incomplete Freund's adjuvant (IFA); an insulin altered peptide ligand is also in clinical trials (Box 1). Although it is unclear to what extent HSP is an autoantigen, clinical trials with DiaPep 277 are in progress (Box 1). In an approach to test whether GAD65 might modulate GAD65 autoreactivity in humans, a Phase II study with recombinant human GAD65 formulated with alum was conducted in LADA patients [59]. The study was conducted as a randomized, double-blind, placebo-controlled, dose-escalation clinical trial in a total of 47 LADA patients, who received placebo or 4, 20, 100 or 500 μ g alum-formulated GAD65 subcutaneously initially and then four weeks later. None of the patients showed significant study-related adverse events. Fasting c-peptide levels at 24 weeks post-administration of alum-formulated GAD65 were increased compared to placebo in the 20 μ g but not in the other dose groups. It was of interest that this increase in c-peptide correlated with an increase in the $CD4^+CD25^+ : CD4^+CD25^-$ cell ratio. The 20 μ g dose group, together with placebo, are currently being tested in two Phase IIb clinical trials (one in 160 LADA patients and one in 76 new onset type 1 diabetes patients) (Box 1).

The approach to antigen-specific immunomodulation will, however, require additional precautions because GAD65Ab is observed primarily in subjects with DR3-DQA1*0501-B1*0201 and IA-2A and IAA are observed primarily in DR4-DQA1*0301-B1*0302 subjects [25,60]. In addition, IAA is more often detected in subjects with the high risk INS VNTR (insulin gene variable number of tandem repeats) genotype [25,61]. Future immunomodulation trials might therefore have to ascertain subjects

based on HLA and other type 1 diabetes genetic risk factors to fully dissect the possible effects of individual autoantigens. The residual c-peptide level at entry to the trial (i.e. when the first anti-CD3 injection is given) is an important determinant to identify a responder subgroup in the recent anti-CD3 trial [49]. In addition, several recent studies on epitope-specific autoantibody isoforms and isotypes [62–64] indicate that the predictive value of an autoantibody test for type 1 diabetes can be improved. These refined autoantibody tests, known to reflect T-cell activity, should therefore also be included as surrogate endpoints in future autoantigen-specific immunomodulation trials. Provided it is safe, a therapy that combines anti-CD3 [49] with one or several autoantigens might be contemplated [65].

Summary

Immunosuppression or immunomodulation therapy in type 1 diabetes shows great promise but does not yet replace the life-saving insulin treatment. Recent trials with insulin or nicotinamide, modeled following data in the NOD mouse, have failed. This is not the case for CD3 antibody therapy, which demonstrated efficacy both in overtly type 1 diabetic patients and NOD mice. Current clinical trials, or plans for such trials, tend to revert to immunosuppression therapy with agents already used in transplantation. Few of these trials incorporate insulin, GAD65 or IA-2 as autoantigens. A subanalysis showed a reduction of type 1 diabetes in risk subjects with high IAA levels, and will therefore be repeated. Studies are therefore needed to establish the safety of immunomodulation combined with autoantigens, to explore the possibility that tolerance induction might reduce persistent autoantibodies and preserve β -cell function.

Safety is the major concern in these approaches. Perhaps anti-CD3 monoclonal antibodies, which in combination with immunomodulatory reagents, might yield a combination therapy effective enough to induce immunological tolerance. Further standardization and proficiency testing of epitope-specific isotype and subtype autoantibody assays will be needed. For now, these assays will serve as surrogate markers of T-cell activity until standardized disease or autoantigen-specific human T-cell assays have been developed.

References

- Wallenstein, M. *et al.* (1988) Factors influencing the magnitude, duration, and rate of fall of B-cell function in type 1 (insulin-dependent) diabetes children followed for two years from their clinical diagnosis. *Diabetologia* 31, 664–669
- Falorni, A. and Brozzetti, A. (2005) Diabetes-related antibodies in adult diabetic patients. *Best Pract. Res. Clin. Endocrinol. Metab.* 19, 119–133
- Davis, T.M. *et al.* (2005) Islet autoantibodies in clinically diagnosed type 2 diabetes: prevalence and relationship with metabolic control (UKPDS 70). *Diabetologia* 48, 695–702
- Klöppel, G. *et al.* (1984) Morphometric evidence for a striking β -cell reduction at the clinical onset of type 1 diabetes. *Virchows Arch. A Pathol. Anat. Histopathol.* 403, 441–452
- Pipeleers, D. and Ling, Z. (1992) Pancreatic β cells in insulin-dependent diabetes. *Diabetes Metab. Rev.* 8, 209–227
- Beard, M.E. *et al.* (2002) Is type 1 diabetes transmissible by bone marrow allograft? *Diabetes Care* 25, 799–800
- Lampeter, E.F. *et al.* (1998) Transfer of diabetes type 1 by bone-marrow transplantation. *Lancet* 351, 568–569
- Martin, S. *et al.* (2001) Development of type 1 diabetes despite severe hereditary B-lymphocyte deficiency. *N. Engl. J. Med.* 345, 1036–1040
- Toma, A. *et al.* (2005) Recognition of a subregion of human proinsulin by class I-restricted T cells in type 1 diabetic patients. *Proc. Natl. Acad. Sci. U. S. A.* 102, 10581–10586
- Panagiotopoulos, C. *et al.* (2003) Identification of a β -cell-specific HLA class I restricted epitope in type 1 diabetes. *Diabetes* 52, 2647–2651
- Dromei, J.A. *et al.* (2004) Mapping of epitopes for autoantibodies to the type 1 diabetes autoantigen IA-2 by peptide phage display and molecular modeling: overlap of antibody and T cell determinants. *J. Immunol.* 172, 4084–4090
- Lieberman, S.M. *et al.* (2003) Identification of the β cell antigen targeted by a prevalent population of pathogenic CD8⁺ T cells in autoimmune diabetes. *Proc. Natl. Acad. Sci. U. S. A.* 100, 8384–8388
- Nagata, M. *et al.* (2004) Detection of autoreactive T cells in type 1 diabetes using coded autoantigens and an immunoglobulin-free cytokine ELISPOT assay: report from the fourth immunology of diabetes society T cell workshop. *Ann. N. Y. Acad. Sci.* 1037, 10–15
- Novak, E.J. *et al.* (2001) Activated human epitope-specific T cells identified by class II tetramers reside within a CD4high, proliferating subset. *Int. Immunol.* 13, 799–806
- Reijonen, H. *et al.* (2002) Detection of GAD65-specific T-cells by major histocompatibility complex class II tetramers in type 1 diabetic patients and at-risk subjects. *Diabetes* 51, 1375–1382
- Brooks-Worrell, B. *et al.* (2001) Intermolecular antigen spreading occurs during the preclinical period of human type 1 diabetes. *J. Immunol.* 166, 5265–5270
- Schloot, N.C. *et al.* (2003) Comparison of cytokine ELISPOT assay formats for the detection of islet antigen autoreactive T cells. Report of the third immunology of diabetes society T-cell workshop. *J. Autoimmun.* 21, 365–376
- Bingley, P.J. *et al.* (2003) Diabetes antibody standardization program: first assay proficiency evaluation. *Diabetes* 52, 1128–1136
- Kent, S.C. *et al.* (2005) Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. *Nature* 435, 224–228
- Notkins, A.L. and Lernmark, Å. (2001) Autoimmune type 1 diabetes: resolved and unresolved issues. *J. Clin. Invest.* 108, 1247–1252
- Krischer, J.P. *et al.* (2003) Screening strategies for the identification of multiple antibody-positive relatives of individuals with type 1 diabetes. *J. Clin. Endocrinol. Metab.* 88, 103–108
- Grubin, C.E. *et al.* (1994) A novel radioligand binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. *Diabetologia* 37, 344–350
- Verge, C.F. *et al.* (1998) Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. *Diabetes* 47, 1857–1866
- Mire-Sluis, A.R. *et al.* (2000) The World Health Organization international collaborative study for islet cell antibodies. *Diabetologia* 43, 1282–1292
- Graham, J. *et al.* (2002) Genetic effects on age-dependent onset and islet cell autoantibody markers in type 1 diabetes. *Diabetes* 51, 1346–1355
- Gottlieb, P.A. and Eisenbarth, G.S. (1998) Diagnosis and treatment of pre-insulin dependent diabetes. *Annu. Rev. Med.* 49, 391–405
- Leslie, D. *et al.* (2001) Autoantibodies as predictors of disease. *J. Clin. Invest.* 108, 1417–1422
- Notkins, A.L. (2004) Type 1 diabetes as a model for autoantibodies as predictors of autoimmune diseases. *Autoimmun. Rev.* 3 (Suppl. 1), S7–S9
- Eisenbarth, G.S. (2004) Prediction of type 1 diabetes: the natural history of the prediabetic period. *Adv. Exp. Med. Biol.* 552, 268–290
- Ziegler, A.G. *et al.* (1999) Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study. *Diabetes* 48, 460–468
- Barker, J.M. *et al.* (2004) Prediction of autoantibody positivity and progression to type 1 diabetes: Diabetes Autoimmunity Study in the Young (DAISY). *J. Clin. Endocrinol. Metab.* 89, 3896–3902

- 32 Carmichael, S.K. *et al.* (2003) Prospective assessment in newborns of diabetes autoimmunity (PANDA): maternal understanding of infant diabetes risk. *Genet. Med.* 5, 77–83
- 33 Kupila, A. *et al.* (2002) Genetic risk determines the emergence of diabetes-associated autoantibodies in young children. *Diabetes* 51, 646–651
- 34 DPT-1. (2002) Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N. Engl. J. Med.* 346, 1685–1691
- 35 Gale, E.A. *et al.* (2004) European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. *Lancet* 363, 925–931
- 36 Nerup, J. *et al.* (1974) HL-A antigens and diabetes mellitus. *Lancet* 2, 864–866
- 37 Bottazzo, G.F. *et al.* (1974) Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* 2, 1279–1283
- 38 Skyler, J.S. (1987) Immune intervention studies in insulin-dependent diabetes mellitus. *Diabetes Metab. Rev.* 3, 1017–1035
- 39 Skyler, J. and Marks, J. (1996) Immune Intervention. In *Diabetes Mellitus* (LeRoth, D. *et al.*, eds), pp. 402–408, Lippincott-Raven
- 40 Ludvigsson, J. *et al.* (1983) Plasmapheresis in the initial treatment of insulin-dependent diabetes mellitus in children. *BMJ* 286, 176–178
- 41 Sundkvist, G. *et al.* (1994) Islet cell antibodies (ICA) but not glutamic acid decarboxylase antibodies (GAD65-Ab) are decreased by plasmapheresis in patients with newly diagnosed insulin-dependent diabetes mellitus (IDDM). *J. Clin. Endocrinol. Metab.* 78, 1159–1165
- 42 The Canadian–European Randomized Control Trial Group. (1988) Cyclosporin-induced remission of IDDM after early intervention. Association of 1 year of cyclosporin treatment with enhanced insulin secretion. *Diabetes* 37, 1574–1582
- 43 Bougneres, P.F. *et al.* (1988) Factors associated with early remission of type 1 diabetes in children treated with cyclosporine. *N. Engl. J. Med.* 318, 663–670
- 44 Feutren, G. *et al.* (1986) Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet* 2, 119–124
- 45 Parving, H.H. *et al.* (1999) Cyclosporine nephrotoxicity in type 1 diabetic patients. A 7-year follow-up study. *Diabetes Care* 22, 478–483
- 46 Assan, R. *et al.* (2002) Normal renal function 8 to 13 years after cyclosporin A therapy in 285 diabetic patients. *Diabetes Metab. Res. Rev.* 18, 464–472
- 47 Raz, I. *et al.* (2005) Immune modulation for prevention of type 1 diabetes mellitus. *Trends Biotechnol.* 23, 128–134
- 48 Herold, K.C. *et al.* (2002) Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N. Engl. J. Med.* 346, 1692–1698
- 49 Keymeulen, B. *et al.* (2005) Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N. Engl. J. Med.* 352, 2598–2608
- 50 Atkinson, M.A. (2005) Thirty years of investigating the autoimmune basis for type 1 diabetes: why can't we prevent or reverse this disease? *Diabetes* 54, 1253–1263
- 51 Roep, B.O. *et al.* (2004) Satisfaction (not) guaranteed: re-evaluating the use of animal models of type 1 diabetes. *Nat. Rev. Immunol.* 4, 989–997
- 52 Kaufman, D.L. *et al.* (1993) Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature* 366, 69–72
- 53 Tisch, R. *et al.* (1993) Immune response to glutamic acid decarboxylase correlates with insulinitis in non-obese diabetic mice. *Nature* 366, 72–75
- 54 Chatenoud, L. (2003) CD3-specific antibody-induced active tolerance: from bench to bedside. *Nat. Rev. Immunol.* 3, 123–132
- 55 Schultze, J.L. *et al.* (2004) DCs and CD40-activated B cells: current and future avenues to cellular cancer immunotherapy. *Trends Immunol.* 25, 659–664
- 56 Jager, E. *et al.* (2003) Antigen-specific immunotherapy and cancer vaccines. *Int. J. Cancer* 106, 817–820
- 57 Berntorp, E. *et al.* (2000) Immune tolerance induction and the treatment of hemophilia. Malmö protocol update. *Haematologica* 85(10 Suppl.), 48–50
- 58 Skyler, J.S. *et al.* (2005) Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial – Type 1. *Diabetes Care* 28, 1068–1076
- 59 Agardh, C.D. *et al.* (2005) Clinical evidence for safety of GAD65 immunomodulation in adult-onset autoimmune diabetes. *J. Diabetes Complications* 19, 238–246
- 60 Knip, M. *et al.* (2002) Humoral β -cell autoimmunity in relation to HLA-defined disease susceptibility in preclinical and clinical type 1 diabetes. *Am. J. Med. Genet.* 115, 48–54
- 61 Walter, M. *et al.* (2003) IDDM2/insulin VNTR modifies risk conferred by IDDM1/HLA for development of Type 1 diabetes and associated autoimmunity. *Diabetologia* 46, 712–720
- 62 Achenbach, P. *et al.* (2004) Stratification of type 1 diabetes risk on the basis of islet autoantibody characteristics. *Diabetes* 53, 384–392
- 63 Hoppu, S. *et al.* (2004) GAD65 antibody isotypes and epitope recognition during the prediabetic process in siblings of children with type 1 diabetes. *Clin. Exp. Immunol.* 136, 120–128
- 64 Hoppu, S. *et al.* (2004) IA-2 antibody epitopes and isotypes during the prediabetic process in siblings of children with type 1 diabetes. *J. Autoimmun.* 23, 361–370
- 65 Lernmark, A. (2005) Type 1 diabetes – does suppressing T cells increase insulin? *N. Engl. J. Med.* 352, 2642–2644

AGORA initiative provides free agriculture journals to developing countries

The *Health Internetwork Access to Research Initiative* (HINARI) of the WHO has launched a new community scheme with the UN Food and Agriculture Organization.

As part of this enterprise, Elsevier has given 185 journals to *Access to Global Online Research in Agriculture* (AGORA). More than 100 institutions are now registered for the scheme, which aims to provide developing countries with free access to vital research that will ultimately help increase crop yields and encourage agricultural self-sufficiency.

According to the Africa University in Zimbabwe, AGORA has been welcomed by both students and staff. 'It has brought a wealth of information to our fingertips' says Vimbai Hungwe. 'The information made available goes a long way in helping the learning, teaching and research activities within the University. Given the economic hardships we are going through, it couldn't have come at a better time.'

For more information visit:
<http://www.healthinternetwork.net>