

Perspectives in Diabetes

Guidelines for Intervention Trials in Subjects With Newly Diagnosed Type 1 Diabetes

Carla J. Greenbaum¹ and Leonard C. Harrison,² on behalf of the Immunology of Diabetes Society

Type 1, or insulin-dependent diabetes, is an autoimmune disease that culminates in the destruction of insulin-producing β -cells in the islets of the pancreas. Studies in the nonobese diabetic (NOD) mouse model of spontaneous type 1 diabetes provide “proof-of-concept” that the disease is preventable (1). People with type 1 diabetes and their relatives, researchers, government, and industry are eager to move forward and test candidate intervention/prevention therapies in humans. Such therapies may entail risks, including accelerated loss of β -cell function, malignancy, and infection. Scientifically and ethically, investigators are obliged to maximize the information gained from intervention trials and minimize risks. One way of achieving this is by standardizing trial protocols. Standardization of islet autoantibody assays (2–13) and of the intravenous glucose tolerance test for measuring first-phase insulin response (14–18) has been a major advance, allowing stratification for disease risk among relatives. The literature on intervention trials in newly diagnosed type 1 diabetic patients (19–44) reveals that entry criteria, trial design and duration, and outcome measures differ considerably. Adoption of standardized protocols would permit comparative and pooled data analysis and facilitate evaluation of potential therapies.

Our purpose here is to highlight issues pertaining to trial variables and suggest ways of standardizing protocols for phase I and II intervention trials in newly diagnosed patients. These issues will be discussed under three major headings: trial subjects, trial design, and trial outcome measures.

SUBJECTS: INCLUSION CRITERIA

Diagnosis of diabetes

Background. Type 1 diabetes can have different clinical presentations that presumably reflect the nature of the underlying disease pathology, to which we have no direct

From the ¹Benaroya Research Institute at Virginia Mason, Seattle, Washington; and the ²Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia.

Address correspondence and reprint requests to Carla J. Greenbaum, Benaroya Research Institute at Virginia Mason, Seattle, WA 98101. E-mail: cjgreen@vmresearch.org.

Received for publication 9 May 2002 and accepted in revised form 7 January 2003.

AUC, area under curve; MMTT, mixed-meal tolerance test.

© 2003 by the American Diabetes Association.

access. Some patients present acutely with dehydration and ketoacidosis, whereas others have minimal or no symptoms (45,46). Natural history studies have indicated that these differences may correlate with the rate of loss of β -cell function and residual β -cell function, determined by genetic (47–49) and other (50–66) factors that modify disease pathology. However, the relationship between the nature of the clinical presentation and the effectiveness of intervention therapy is not known (Table 1).

Proposal

- Define onset of diabetes from time of diagnosis by a physician, based on recognized, e.g., American Diabetes Association, criteria.
- Document the following at clinical presentation: age, sex, pubertal status, family history of diabetes, blood glucose, bicarbonate, presence or absence of ketoacidosis, weight loss, polyuria, polydipsia, HbA_{1c}, islet autoantibodies, insulin requirement, and HLA typing.

Age

Background. The natural history of pre- and postclinical type 1 diabetes varies with age. Specifically, the rate of β -cell destruction is inversely related to age (50–53,58). This age effect is directly associated with the number of susceptibility HLA class II (e.g., DR 3,4; DQ 2,8) (47,48,67) and class I (e.g., A24) (49) alleles. The more susceptibility alleles there are, the younger the age of onset and diagnosis, with a more autoaggressive immune response reflected by the number of islet antibodies (68–70). Therefore, the requirement for effective intervention treatment is likely to be more demanding in younger subjects. On the other hand, a slower rate of β -cell destruction in older subjects may indicate a wider window of opportunity for intervention; although, if the process was “regulated,” it would be important that intervention treatment did not jeopardize this.

Although an upper age limit may delineate classic type 1 diabetes from slowly progressive type 1 diabetes or latent autoimmune diabetes of adults (71–73), the combination of clinical type 2 diabetes and autoantibodies may still occur in children and younger adults (74). Age is also an issue with respect to consent and recruitment.

Proposal

- Match subjects in treatment and control groups as closely as possible for age.
- In phase I trials, enroll only subjects ≥ 18 years of age.

TABLE 1
Inclusion criteria

Diagnosis of diabetes	● According to American Diabetes Association criteria
Age	● Phase I trials \geq age 18 years ● Phase II, and III trials \leq age 35 years
Autoantibodies	● One of four to GAD65, insulin (if on insulin <2 weeks), IA2, or ICA
Start of therapy in relationship to diabetes diagnosis	● Baseline MMTT peak C-peptide ≥ 0.2 pmol/l ● If early-onset trial, subjects to be enrolled between 2 and 12 weeks from diagnosis

- Limit entry to subjects aged <35 years.

Autoantibodies

Background. Type 1A diabetes is an immune-mediated disease resulting in loss of β -cells. During the past several decades, islet autoantibodies to the GAD65 isoform (GADAb), tyrosine phosphatase-like insulinoma antigen IA2 (IA2Ab), and insulin (IAA) have been identified in individuals at risk for and presenting with clinical disease. Although up to 10% of patients presenting with clinical type 1 diabetes are islet autoantibody-negative (64) and ~ 10 –15% of patients with clinical type 2 diabetes are autoantibody-positive (71–74), autoantibody measurements remain the best indication that diabetes is immune mediated. Most would agree that the presence of one or more islet autoantibodies (GADAb, IA2Ab, or IAA, measured within 2 weeks of diabetes diagnosis) indicates immune-mediated disease and is a sufficient criterion for entry. More controversial is whether the presence of ICA alone is also a sufficient criterion for entry. Measurement of ICA by immunofluorescence requires a larger sample and is more difficult to perform than newer radioimmunoassays developed for GADAb, IA2Ab, and IAA. In subjects at risk for diabetes, the presence of ICA or any one autoantibody alone may not confer sufficient risk for entry to prevention trials; however, in subjects *with* diabetes, ICA is a marker of immune-mediated disease. Therefore, a patient with diabetes confirmed positive for ICA in the absence of the other three autoantibodies should also be eligible for study enrollment.

Proposal

- Subjects should have at least one of four islet autoantibodies: to GAD65, insulin (if on insulin treatment <2 weeks), IA2, or ICA.

Time from diagnosis

Background. In general, time from diagnosis is inversely related to C-peptide secretion. However, data from the Diabetes Control and Complications Trial and other studies indicate that some subjects with type 1 diabetes continue to have residual C-peptide, even 5 years after diagnosis (50,53,75–78). Time from diagnosis is therefore

not necessarily an accurate index of residual β -cell function. Additionally, measurement of C-peptide secretion when diabetes is poorly controlled is unreliable (see below).

Two models of the disease have been proposed: in one, clinical onset occurs on a continuum of the immune assault, with β -cell function finally being inadequate to maintain normoglycemia; in the other, the process of β -cell injury becomes abruptly destructive, heralding clinical diagnosis (65,66,79,80). In the latter, initiation of treatment within a short timeframe would be essential. In addition, data from cyclosporin trials suggest that early treatment is beneficial. Thus, investigators may wish to enroll subjects relatively soon after diagnosis in “early-onset trials.”

Such early-onset trials should be distinguished from those in which the only entry criterion is residual C-peptide secretion. In the latter, matching for time from diagnosis where there is a small number of subjects or randomizing where there is a larger number of subjects would be particularly important to obviate the potential problem of enrolling “survivors” with persisting C-peptide secretion.

Proposal

- Document peak C-peptide of ≥ 0.2 pmol/l after a liquid mixed-meal tolerance test (MMTT) (Sustacal/Boost). This baseline test should only be done after the subject is metabolically stable (at least 2 weeks after diagnosis).
- Studies defined as early-onset trials should include only subjects <12 weeks from diagnosis. Otherwise, no specific time from diagnosis is recommended.

TRIAL DESIGN

Number of subjects

Background. Phase I and II studies are often not large enough to stratify subjects according to important variables (Table 2).

Proposal

- Aim to include sufficient numbers of subjects to enable stratification in phase III trials. For smaller trials, collect

TABLE 2
Trial design

Number of subjects	● Aim to include sufficient numbers of subjects to enable stratification
Duration of trial	● Efficacy should be evaluated at a minimum of 2 years
Factors that influence outcome measures	● Aim for tight control (e.g., as close to normal HbA _{1c} as possible without causing hypoglycemia) ● Randomize, placebo-control, and double-mask subjects in phase II and III trials

standardized raw data on all subjects for later combined analysis. Document age, sex, pubertal status, family history of diabetes, time from diagnosis, nature of clinical presentation (see above), HLA, baseline immune marker, and C-peptide status.

Duration of trial

Background. It is assumed that mixed meal- or glucagon-stimulated C-peptide falls after diagnosis, and power calculations may be predicated on intervention reducing the rate of fall. However, data from control arms of trials in recently diagnosed adults indicate there may be little or no fall in C-peptide over the first year (42,43). Therefore, evaluation out to 1 year after either diagnosis or treatment initiation may fail to accurately reflect outcome, particularly in adults in whom there may only be a minimal fall in C-peptide over this period. Evaluation at this time may, however, provide short-term safety data.

Proposal

- Evaluate treatment for at least 2 years, particularly in adults; 1 year may be appropriate for safety.

Factors that influence outcome measures

Background. Diabetes treatment (24,36,78,81,82), physical activity, diet, time of testing, and other variables influence diabetes control and outcome measures.

The standard of care for people with diabetes is “tight” control (i.e., $HbA_{1c} < 7\%$) (83). In some intervention trials, subjects have been taken off insulin when euglycemia was achieved (84–86). It remains unknown whether continuing insulin therapy even during the honeymoon phase is beneficial, but indirect evidence suggests it is (78,81,82). The failure of parenteral insulin to prevent diabetes does not indicate that insulin treatment is without benefit in subjects with diabetes. Thus, unless a subject has reached an “insulin-free” end point (see below), insulin treatment should be continued.

Proposal

- Aim to standardize variables that could influence diabetes control and/or outcome measures.
- Randomize subjects in phase II and III trials.
- Aim to placebo control and double mask.
- Mask blood samples before analysis.
- Review safety and other data by external committee (e.g., a data safety monitoring board), with code broken to investigators and subjects if necessary for reasons of safety. Otherwise, do not break codes for data analysis until termination of the trial.
- Aim for tight control (e.g., as close to normal HbA_{1c} as possible without causing hypoglycemia).
- Continue insulin treatment whenever possible (avoiding hypoglycemia) unless subject has reached an insulin-free end point (see below).

TRIAL OUTCOME MEASURES

Metabolic tests

Background. Several tests can be used to evaluate β -cell function. C-peptide in healthy subjects can be stimulated by intravenous, intramuscular, or subcutaneous glucagon; intravenous sulfonylurea; intravenous glucagon-like pep-

TABLE 3
Outcome measures

Metabolic tests	• 2-h MMTT every 3 months
Immune tests	• Standardized autoantibodies
Primary outcome	• Difference in 2-h AUC C-peptide between treated and control groups
Secondary outcomes	• Insulin dose per kilogram, HbA_{1c} level

ptide 1; intravenous or oral amino acids; intravenous or oral glucose; or a mixed meal (87–92). During intervention with cyclosporin, subjects with type 1 diabetes had C-peptide responses to a MMTT at a time when intravenous glucose and glucagon responses were absent (93). Most studies have only evaluated the C-peptide response to an oral mixed meal over 2 h, although it has been suggested that a 4-h test may provide additional useful information, because many subjects with impaired β -cell function do not reach a peak C-peptide value during 2 h. Unfortunately, a 4-h MMTT can be difficult to perform, particularly in subjects with minimal residual function due to hypo- and hyperglycemia occurring during the test. Alternatively, intravenous glucagon-stimulated C-peptide has been used in new-onset trials. However, there is limited information regarding the relationship between MMTT and glucagon test results (92,94,95), and there are no data indicating that one test is preferable to the other. Nonetheless, for the purpose of having standardized end points, the MMTT is the recommended test. If investigators choose to perform intravenous glucagon stimulation of C-peptide, a MMTT should be performed in addition at least at baseline and annually to obtain comparative data (Table 3).

There are little published data on conditions that affect C-peptide stimulation tests in patients with established type 1 diabetes. An important consideration is the control of diabetes in the peri-test period. Although one study reported no effect of exogenous insulin on the MMTT (96), most protocols advise withholding insulin before the test. Should this only apply to short-acting insulin? What about insulin via the pump? The importance of the prevailing blood glucose level on stimulated C-peptide remains controversial. Some studies suggest no effect (87), whereas others indicate that the test is only valid in the absence of hypoglycemia (94,97) or hyperglycemia (98–100).

Proposal

- Evaluate stimulated C-peptide with the liquid MMTT on a quarterly basis.
- Administer evening insulin as usual but withhold morning insulin of any type. If on the pump, continue the basal rate but withhold the bolus. Conduct the test only if fasting blood glucose is 4–11.1 mmol/l (72–200 mg/dl).

Immune tests

Background. Antibodies (titer, isotypes, IgG subclasses, and epitope specificity) and T-cell responses (proliferation, activation markers, and cytokine production) may change in response to intervention therapy and therefore provide important mechanistic “surrogate marker” information. However, autoantibody changes cannot be used as an outcome measure because the relationship between changes in these markers and therapeutic benefit is un-

known. For example, in the cyclosporin trials, islet antibody levels did not correlate with benefit (101), whereas remission of Graves' hyperthyroidism (an autoantibody-mediated disease) has been associated with a decrease in autoantibody levels (102,103).

The place of markers such as IgG autoantibody subclasses (104,105) and islet antigen-reactive T-cell responses (106–108) is not yet clear. Assays for these cells are being evaluated by Immunology of Diabetes Society Workshops (109). T-cell assays require substantial improvement so that reproducible, quantitative, and qualitative responses can be measured.

Proposal

- Measure islet autoantibodies and freeze sera/plasma for future studies. Consider freezing blood mononuclear cells for future analysis.
- Evaluate immune markers in regard to HLA types.

Primary and secondary outcomes

Background. Studies have reported changes in fasting, peak, and area under curve (AUC) C-peptide values over time. It remains unclear which is most useful. In addition, it is not known whether C-peptide expressed as a function of blood glucose is more reliable. There are prepubertal versus postpubertal/age differences in C-peptide that are often not taken into account.

Withdrawal of insulin should be done only in the context of preventing hypoglycemia, not as a primary goal of treatment. However, in some subjects, therapy may result in restoration of a euglycemic insulin-free state.

Proposal

- Define the primary outcome as a significant difference in the 2-h AUC C-peptide response between treated and control groups over time. In addition, analyze incremental and peak C-peptide responses. Additional analysis, such as time to peak C-peptide response or 4-h AUC for C-peptide, may be an appropriate exploratory outcome.
- Define secondary outcomes as insulin dose per kilogram and HbA_{1c} level.
- Subjects at least 1 year from diagnosis on limited amounts of insulin with normal HbA_{1c} levels on two occasions 3 months apart are potentially "insulin-free." However, before withdrawal of chronic insulin therapy, documentation of normal glucose response is needed. These subjects should undergo a standard oral glucose tolerance test after not receiving insulin for 3 days. The presence of normal glucose tolerance under these conditions indicates an insulin-free state, and chronic insulin administration can be discontinued. Close follow-up with repeated HbA_{1c} and glucose tolerance tests are recommended, with reinstatement of insulin if abnormalities are present.

CONCLUSION

These Immunology of Diabetes Society guidelines have been developed to facilitate comparison of intervention therapies. Development and validation of novel assay technologies as well as new data on alternative outcome measures will undoubtedly require modifications to these recommendations in the future, but the principle that

standardization of clinical intervention trials benefits patients, families, and investigators will continue to underlie these efforts.

ACKNOWLEDGMENTS

C.J.G. was supported in part by grants from the Paul G. Allen Foundation Clinical Scholars Program, the Buse Diabetes Clinical Research Chair, and the Juvenile Diabetes Research Foundation Center. L.C.H. was supported by a Juvenile Diabetes Research Foundation Center grant and by the National Health and Medical Research Council of Australia.

The following individuals provided comments on these guidelines: Desmond A. Schatz, George S. Eisenbarth, Jerry P. Palmer, Kevan C. Herold, Paolo Pozzilli, Edwin A. Gale, Hubert Kolb, Olov Rolandsson, Didac Mauricio, Peter G. Colman, and Spiros Fourlanos.

REFERENCES

1. Atkinson MA, Leiter EH: The NOD mouse model of type 1 diabetes: as good as it gets? *Nat Med* 5:601–604, 1999
2. Bottazzo G, Gleichmann H: Immunology and Diabetes Workshops: report of the First International Workshop on the Standardization of Cytoplasmic Islet Cell Antibodies. *Diabetologia* 29:125–126, 1986
3. Bonifacio E, Lernmark A, Dawkins RL: Serum exchange and use of dilutions have improved precision measurement of islet cell antibodies. *J Immunologic Methods* 106:83–88, 1988
4. Boitard C, Bonifacio E, Bottazzo G, Gleichmann H, Molenaar J: Immunology and Diabetes Workshop: report on the Third International (Stage 3) Workshop on the Standardization of Cytoplasmic Islet Cell Antibodies. *Diabetologia* 31:451–452, 1988
5. Wilkin T, Palmer J, Kurta A, Bonifacio E, Diaz J-L: The Second International Workshop on the Standardization of Insulin Autoantibody (IAA) Measurement. *Diabetologia* 31:449–450, 1988
6. Bonifacio E, Boitard C, Gleichmann H, Shattock MA, Molenaar JL, Bottazzo GF: Assessment of precision, concordance, specificity, and sensitivity of islet cell antibody measurement in 41 assays. *Diabetologia* 33:731–736, 1990
7. Lernmark A, Molenaar JL, van Beers WA, Yamaguchi Y, Nagataki S, Ludvigsson J, Maclaren NK: The Fourth International Serum Exchange Workshop to Standardize Cytoplasmic Islet Cell Antibodies: The Immunology and Diabetes Workshops and Participating Laboratories. *Diabetologia* 34:534–535, 1991
8. Greenbaum C, Palmer J, Nagataki S, Yamaguchi Y, Molenaar J, VanBeers W, Maclaren N, Lernmark A: Improved specificity of ICA assays in the Fourth International Immunology of Diabetes Serum Exchange Workshop. *Diabetes* 41:1570–1574, 1992
9. Greenbaum CJ, Palmer JP, Kuglin B, Kolb H: Insulin autoantibodies measured by radioimmunoassay methodology are more related to insulin-dependent diabetes mellitus than those measured by enzyme-linked immunosorbent assay: results of the Fourth International Workshop on the Standardization of Insulin Autoantibody Measurement. *J Clin Endocrinol Metab* 74:1040–1044, 1992
10. Greenbaum C, Wilkin T, Palmer J: Fifth International Serum Exchange Workshop for Insulin Autoantibody (IAA) Standardization. *Diabetologia* 35:798–800, 1992
11. Schmidli RS, Colman PG, Bonifacio E, Bottazzo GF, Harrison LC: High level of concordance between assays for glutamic acid decarboxylase antibodies: the First International Glutamic Acid Decarboxylase Antibody Workshop. *Diabetes* 43:1005–1009, 1994
12. Bingley P: Interactions of age, islet cell antibodies, insulin autoantibodies, and first-phase insulin response in predicting risk of progression to IDDM in ICA+ relatives: the ICARUS data set. *Diabetes* 45:1720–1728, 1996
13. Verge CF, Stenger D, Bonifacio E, Colman PG, Pilcher C, Bingley PJ, Eisenbarth GS: 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, 1998
14. Colman PG, Stewart V, Kean J, Koschmann M, Alford F, Ward G, Deam D, Harrison LC: Comparison of two commonly used standard intravenous glucose tolerance tests. *Diabetes Care* 15:1053–1055, 1992
15. Koschmann M, Alford FP, Ward GM, Walters J, Colman PG, Harrison LC:

- Reproducibility of estimating first phase insulin responses to intravenous glucose. *Diab Nutr Metab* 5:73-79, 1992
16. Bingley PJ, Colman P, Eisenbarth GS, Jackson RA, McCulloch DK, Riley WJ, Gale EA: Standardization of IVGTT to predict IDDM. *Diabetes Care* 15:1313-1316, 1992
 17. McCulloch D, Bingley P, Colman P, Jackson R, Gale E: Comparison of bolus and infusion protocols for determining acute insulin response to intravenous glucose in normal humans. *Diabetes Care* 16:911-915, 1993
 18. McNair PD, Colman PG, Alford A, Harrison LC: Reproducibility of the first phase insulin response to intravenous glucose is not improved by retrograde cannulation and arterialization or the use of a lower glucose dose. *Diabetes Care* 18:1168-1173, 1995
 19. Harrison LC, Colman PG, Dean B, Baxter R, Martin FI: Increase in remission rate in newly diagnosed type 1 diabetic subjects treated with azathioprine. *Diabetes* 34:1306-1308, 1985
 20. Silverstein J, Maclaren N, Riley W, Spillar R, Radjenovic D, Johnson S: Immunosuppression with azathioprine and prednisone in recent-onset insulin-dependent diabetes mellitus. *N Engl J Med* 319:599-604, 1988
 21. Canadian-European Randomized Trial Group: Cyclosporin-induced remission of IDDM: after early intervention: association of 1 yr of cyclosporin treatment with enhanced insulin secretion. *Diabetes* 37:1574-1582, 1988
 22. Cook JJ, Hudson I, Harrison LC, Dean B, Colman PG, Werther GA, Warne GL, Court JM: Double-blind controlled trial of azathioprine in children with newly diagnosed type 1 diabetes. *Diabetes* 38:779-783, 1989
 23. Mendola G, Casamitjana R, Gomis R: Effect of nicotinamide therapy upon B-cell function in newly diagnosed type I (insulin-dependent) diabetic patients. *Diabetologia* 32:160-162, 1989
 24. Shah S, Malone J, Simpson N: A randomized trial of intensive insulin therapy in newly diagnosed insulin-dependent diabetes mellitus. *N Engl J Med* 320:550-554, 1989
 25. Vague P, Picq R, Bernal M, Lassman-Vague V, Vialettes B: Effect of nicotinamide treatment on the residual insulin secretion in type 1 (insulin-dependent) diabetic patients. *Diabetologia* 32:316-321, 1989
 26. Chase HP, Butler-Simon N, Garg S, McDuffie M, Hoops SL, O'Brien D: A trial of nicotinamide in newly diagnosed patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 33:444-446, 1990
 27. Giordano C, Panto F, Amato MP, Sapienza N, Pugliese A, Galluzzo A: Early administration of an immunomodulator and induction of remission in insulin-dependent diabetes mellitus. *J Autoimmun* 3:611-617, 1990
 28. Secchi A, Pastore MR, Sergi A, Pontiroli AE, Pozza G: Prednisone administration in recent onset type 1 diabetes. *J Autoimmun* 3:593-600, 1990
 29. Skyler JS, Rabinovitch A: Cyclosporine in recent onset type 1 diabetes mellitus: effects on islet beta cell function: Miami Cyclosporine Diabetes Study Group. *J Diabetes Complications* 6:77-88, 1992
 30. Goday A, Pujol-Borrell R, Fernandez J, Casamitjana R, Rios M, Vilardell E, Gomis R: Effects of a short prednisone regime at clinical onset of type 1 diabetes. *Diabetes Res Clin Pract* 20:39-46, 1993
 31. Muir A, Schatz D, Maclaren N: Antigen-specific immunotherapy: oral tolerance and subcutaneous immunization in the treatment of insulin-dependent diabetes. *Diabetes Metab Rev* 9:279-287, 1993
 32. Skyler JS, Lorenz TJ, Schwartz S, Eisenbarth GS, Einhorn D, Palmer JP, Marks JB, Greenbaum C, Saria EA, Byers V: Effects of an anti-CD5 immunconjugate (CD5-plus) in recent onset type 1 diabetes mellitus: a preliminary investigation: the CD5 Diabetes Project Team. *J Diabetes Complications* 7:224-232, 1993
 33. Pozzilli P, Visalli N, Signore A, Baroni MG, Buzzetti R, Cavallo MG, Boccuni ML, Fava D, Gragnoli C, Andreani D: Double blind trial of nicotinamide in recent-onset IDDM (the IMDIAB III study). *Diabetologia* 38:848-852, 1995
 34. Bjork E, Berne C, Kampe O, Wibell L, Oskarsson P, Karlsson FA: Diazoxide treatment at onset preserves residual insulin secretion in adults with autoimmune diabetes. *Diabetes* 45:1427-1430, 1996
 35. Kohnert KD, Hehmke B, Keilacker H, Ziegler M, Emmrich F, Laube F, Michaelis D: Antibody response to islet antigens in anti-CD4/prednisolone immune intervention of type 1 diabetes. *Int J Clin Lab Res* 26:55-59, 1996
 36. Linn T, Ortac K, Laube H, Federlin K: Intensive therapy in adult insulin-dependent diabetes mellitus is associated with improved insulin sensitivity and reserve: a randomized, controlled, prospective study over 5 years in newly diagnosed patients. *Metabolism* 45:1508-1513, 1996
 37. Schnell O, Eisfelder B, Standl E, Ziegler AG: High-dose intravenous insulin infusion versus intensive insulin treatment in newly diagnosed IDDM. *Diabetes* 46:1607-1611, 1997
 38. Coutant R, Landais P, Rosilio M, Johnsen C, Lahlou N, Chatelain P, Carel JC, Ludvigsson J, Boitard C, Bougneres PF: Low dose linomide in type 1 juvenile diabetes of recent onset: a randomised placebo-controlled double blind trial. *Diabetologia* 41:1040-1046, 1998
 39. Elliott JF, Marlin KL, Couch RM: Effect of Bacillus Calmette-Guerin vaccination on C-peptide secretion in children newly diagnosed with IDDM. *Diabetes Care* 21:1691-1693, 1998
 40. Allen HF, Klingensmith GJ, Jensen P, Simoes E, Hayward A, Chase HP: Effect of Bacillus Calmette-Guerin vaccination on new-onset type 1 diabetes: a randomized clinical study. *Diabetes Care* 22:1703-1707, 1999
 41. Buckingham BA, Sandborg CI: A randomized trial of methotrexate in newly diagnosed patients with type 1 diabetes mellitus. *Clin Immunol* 96:86-90, 2000
 42. Chaillous L, Lefevre H, Thivolet C, Boitard C, Lahlou N, Atlan-Gepner C, Bouhanick B, Mogenet A, Nicolino M, Carel JC, Lecomte P, Marechaud R, Bougneres P, Charbonnel B, Sai P: Oral insulin administration and residual beta-cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial: Diabete Insuline Orale group. *Lancet* 356: 545-549, 2000
 43. Pozzilli P, Pitocco D, Visalli N, Cavallo MG, Buzzetti R, Crino A, Spera S, Suraci C, Multari G, Cervoni M, Manca Bitti ML, Matteoli MC, Marietti G, Ferrazzoli F, Cassone Faldetta MR, Giordano C, Sbriglia M, Saruger E, Ghirlanda G: No effect of oral insulin on residual beta-cell function in recent-onset type 1 diabetes (the IMDIAB VII): IMDIAB Group. *Diabetologia* 43:1000-1004, 2000
 44. Vidal J, Fernandez-Balsells M, Semsilo G, Aguilera E, Casamitjana R, Gomis R, Conget I: Effects of nicotinamide and intravenous insulin therapy in newly diagnosed type 1 diabetes. *Diabetes Care* 23:360-364, 2000
 45. Rjasanowski I, Michaelis D, Besch W, Keilacker H, Ziegler B, Hildmann W: Glucose tolerance behaviour before the onset of type 1 (insulin-dependent) diabetes in young people as a predictor of the further course of the disease: a retrospective analysis of 33 cases. *Diabetes Res Clin Pract* 11:107-115, 1991
 46. Greenbaum CJ, Cuthbertson D, Krischer JP, the DPT-1 Study Group: Type 1 diabetes manifested solely by 2-h oral glucose tolerance test criteria. *Diabetes* 50:470-476, 2001
 47. Caillat-Zucman S, Garchon HJ, Timsit J, Assan R, Boitard C, Djilali-Saiah I, Bougneres P, Bach JF: Age-dependent HLA genetic heterogeneity of type 1 insulin-dependent diabetes mellitus. *J Clin Invest* 90:2242-2250, 1992
 48. Tait BD, Harrison LC, Drummond BP, Stewart V, Varney MD, Honeyman MC: HLA antigens and age at diagnosis of insulin-dependent diabetes mellitus. *Hum Immunol* 42:116-122, 1995
 49. Honeyman MC, Harrison LC, Drummond B, Colman PG, Tait BD: Analysis of families at risk for insulin-dependent diabetes reveals that HLA antigens influence progression to preclinical disease. *Mol Med* 1:576-582, 1995
 50. Madsbad S, Faber O, Binder C, McNair P, Christiansen C, Transbol I: Prevalence of residual beta-cell function in insulin-dependent diabetics in relation to age at onset and duration of diabetes. *Diabetes* 27 (Suppl. 1):262-264, 1978
 51. Bonora E, Coscelli C, Butturini U: Residual B-cell function in type 1 (insulin-dependent) diabetes mellitus: its relation to clinical and metabolic features. *Acta Diabetol Lat* 21:375-383, 1984
 52. Sochett EB, Daneman D, Clarson C, Ehrlich RM: Factors affecting and patterns of residual insulin secretion during the first year of type 1 (insulin-dependent) diabetes mellitus in children. *Diabetologia* 30:453-459, 1987
 53. Diabetes Control and Complications Trial Research Group: Effects of age, duration and treatment of insulin-dependent diabetes mellitus on residual beta-cell function: observations during eligibility testing for the Diabetes Control and Complications Trial (DCC). *J Clin Endocrinol Metab* 65:30-36, 1987
 54. Schiffrin A, Suissa S, Poussier P, Guttman R, Weitzner G: Prospective study of predictors of beta-cell survival in type 1 diabetes. *Diabetes* 37:920-925, 1988
 55. Ludvigsson J, Binder C, Mandrup-Poulsen T: Insulin autoantibodies are associated with islet cell antibodies; their relation to insulin antibodies and beta-cell function in diabetic children. *Diabetologia* 31:647-651, 1988
 56. Peig M, Gomis R, Ercilla G, Casamitjana R, Bottazzo GF, Pujol-Borrell R: Correlation between residual beta-cell function and islet cell antibodies in newly diagnosed type I diabetes: follow-up study. *Diabetes* 38:1396-1401, 1989
 57. Couper JJ, Hudson I, Werther GA, Warne GL, Court JM, Harrison LC: Factors predicting residual beta-cell function in the first year after diagnosis of childhood type 1 diabetes. *Diabetes Res Clin Pract* 11:9-16, 1991
 58. Montanya E, Fernandez-Castaner M, Rosel P, Gomez J, Soler J: Age, sex and ICA influence on beta-cell secretion during the first year after the diagnosis of type 1 diabetes mellitus. *Diabet Metab* 17:460-468, 1991

59. Schiffrin A, Suissa S, Weitzner G, Poussier P, Lalla D: Factors predicting course of beta-cell function in IDDM. *Diabetes Care* 15:997-1001, 1992
60. Hramiak IM, Dupre J, Finegood DT: Determinants of clinical remission in recent-onset IDDM. *Diabetes Care* 16:125-132, 1993
61. Yokota I, Shirakawa N, Shima K, Matsuda J, Naito E, Ito M, Kuroda Y: Relationship between GAD antibody and residual beta-cell function in children after overt onset of IDDM. *Diabetes Care* 19:74-75, 1996
62. Bonfanti R, Bazzigaluppi E, Calori G, Riva MC, Viscardi M, Bognetti E, Meschi F, Bosi E, Chiumello G, Bonifacio E: Parameters associated with residual insulin secretion during the first year of disease in children and adolescents with type 1 diabetes mellitus. *Diabet Med* 15:844-850, 1998
63. Sabbah E, Savola K, Kulmala P, Veijola R, Vahasalo P, Karjalainen J, Akerblom HK, Knip M: Diabetes-associated autoantibodies in relation to clinical characteristics and natural course in children with newly diagnosed type 1 diabetes: the Childhood Diabetes in Finland Study Group. *J Clin Endocrinol Metab* 84:1534-1539, 1999
64. Torn C, Landin-Olsson M, Lernmark A, Palmer JP, Arnqvist HJ, Blohme G, Lithner F, Littorin B, Nystrom L, Schersten B, Sundkvist G, Wibell L, Ostman J: Prognostic factors for the course of beta cell function in autoimmune diabetes. *J Clin Endocrinol Metab* 85:4619-4623, 2000
65. Imagawa A, Hanafusa T, Miyagawa J, Matsuzawa Y: A novel subtype of type 1 diabetes mellitus characterized by a rapid onset and an absence of diabetes-related antibodies: Osaka IDDM Study Group. *N Engl J Med* 342:301-307, 2000
66. Imagawa A, Hanafusa T, Miyagawa J, Matsuzawa Y: A proposal of three distinct subtypes of type 1 diabetes mellitus based on clinical and pathological evidence. *Ann Med* 32:539-543, 2000
67. Hoogwerf BJ, Rich SS, Barbosa JJ: Meal-stimulated C-peptide and insulin antibodies in type I diabetic subjects and their nondiabetic siblings characterized by HLA-DR antigens. *Diabetes* 34:440-445, 1985
68. Bingley P: Interactions of age, islet cell antibodies, insulin autoantibodies, and first-phase insulin response in predicting risk of progression to IDDM in ICA+ relatives: the ICARUS data set: Islet Cell Antibody Register Users Study. *Diabetes* 45:1720-1728, 1996
69. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, Chase HP, Eisenbarth GS: Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD and ICA512bdc/IA-2 autoantibodies. *Diabetes* 45:926-933, 1996
70. Torn C, Landin-Olsson M, Lernmark A, Schersten B, Ostman J, Arnqvist HJ, Bjork E, Blohme G, Bolinder J, Eriksson J, Littorin B, Nystrom L, Sundkvist G: Combinations of beta-cell-specific autoantibodies at diagnosis of diabetes in young adults reflects different courses of beta cell damage. *Autoimmunity* 33:115-120, 2001
71. Zimmet PZ, Tuomi T, Mackay IR, Rowley MJ, Knowles W, Cohen M, Lang DA: Latent autoimmune diabetes mellitus in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. *Diabet Med* 11:299-303, 1994
72. Zimmet P, Turner R, McCarty D, Rowley M, Mackay I: Crucial points at diagnosis: type 2 diabetes or slow type 1 diabetes. *Diabetes Care* 22 (Suppl. 2):59-64, 1999
73. Carlsson A, Sundkvist G, Groop L, Tuomi T: Insulin and glucagon secretion in patients with slowly progressing autoimmune diabetes (LADA). *J Clin Endocrinol Metab* 85:76-80, 2000
74. Hathout EH, Thomas W, El-Shahawy M, Nahab F, Mace JW: Diabetic autoimmune markers in children and adolescents with type 2 diabetes. *Pediatrics* 107:E102, 2001
75. Ludvigsson J, Heding LG: Beta-cell function in children with diabetes. *Diabetes* 27 (Suppl. 1):230-234, 1978
76. Faber O: Beta-cell function and diabetic control in insulin dependent diabetes mellitus. *Acta Endocrinol Suppl (Copenh)* 272:73-77, 1985
77. Faber OK, Binder C: C-peptide: an index of insulin secretion. *Diabetes Metab Rev* 2:331-345, 1986
78. Diabetes Control and Complications Trial Research Group: 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. *Ann Intern Med* 128:517-523, 1998
79. Shimada A, Charlton B, Taylor-Edwards C, Fathman CG: Beta-cell destruction may be a late consequence of the autoimmune process in nonobese diabetic mice. *Diabetes* 45:1063-1067, 1996
80. Keller R, Eisenbarth GS: Immunopathogenesis of type 1 diabetes mellitus. In *Immunotherapy of Diabetes and Selected Autoimmune Diseases*. Eisenbarth GS, Ed. Boca Raton, FL, CRC Press, 1989, p. 2-15
81. Kobayashi T, Nakanishi K, Murase T, Kosaka K: Small doses of subcutaneous insulin as a strategy for preventing slowly progressive beta-cell failure in islet cell antibody-positive patients with clinical features of NIDDM. *Diabetes* 45:622-626, 1996
82. 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* 23:314-319, 1997
83. Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977-986, 1993
84. Canadian-European Randomized Control Trial Group: Cyclosporin-induced remission of IDDM after early intervention: association of 1 yr of cyclosporin treatment with enhanced insulin secretion. *Diabetes* 37:1574-1582, 1988
85. Feutren G, Papoz L, Assan R, Vialettes B, Karsenty G, Vexiau P, Du RH, Rodier M, Sirmaj J, Lallemand A: 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, 1986
86. Pozzilli P, Visalli N, Bocconi ML, Baroni MG, Buzzetti-R FE, Signore A, Cavallo MG, Andreani D, Lucentini L, et al.: Randomized trial comparing nicotinamide and nicotinamide plus cyclosporin in recent onset insulin-dependent diabetes (IMDIAB 1): The IMDIAB Study Group. *Diabet Med* 11:98-104, 1994
87. Mirel RD, Ginsberg-Fellner F, Horwitz DL, Rayfield EJ: C-peptide reserve in insulin-dependent diabetes: comparative responses to glucose, glucagon and tolbutamide. *Diabetologia* 19:183-188, 1980
88. Menchini M, Meschi F, Lambiase R, Puzzovio M, Del Guercio MJ, Chiumello G: C-peptide response to arginine stimulation in diabetic children. *J Pediatr* 96:362-366, 1980
89. Scheen AJ, Castillo MJ, Lefebvre PJ: Assessment of residual insulin secretion in diabetic patients using the intravenous glucagon stimulatory test: methodological aspects and clinical applications. *Diabetes Metab* 22:397-406, 1996
90. Rakotoambinina B, Timsit J, Deschamps I, Laborde K, Gautier D, Jos J, Boitard C, Robert JJ: Insulin responses to intravenous glucose, intravenous arginine and a hyperglycaemic clamp in ICA-positive subjects with different degrees of glucose tolerance. *Diabetes Metab* 23:43-50, 1997
91. Sjoberg S, Gunnarsson R, Ostman J: Residual C-peptide production in type I diabetes mellitus: a comparison of different methods of assessment and influence on glucose control. *Acta Med Scand* 214:231-237, 1983
92. Heinze E, Beischer W, Keller L, Winkler G, Teller WM, Pfeiffer EF: C-peptide secretion during the remission phase of juvenile diabetes. *Diabetes* 27:670-676, 1978
93. Skyler JS, Rabinovitch A: Cyclosporine in recent onset type I diabetes mellitus: effects on islet beta cell function: Miami Cyclosporine Diabetes Study Group. *J Diabetes Complications* 2:77-88, 1992
94. Ronnema T: Practical aspects in performing the glucagon test in the measurement of C-peptide secretion in diabetic patients. *Scand J Clin Lab Invest* 46:345-349, 1986
95. Pasquali R, Buratti P, Biso P, Patrono D, Capelli M, Pasqui F, Melchionda N: Estimation of B-cell function by the urinary excretion rate of C-peptide in diabetic patients: comparison with C-peptide response to glucagon and to a mixed meal. *Diabetes Metab* 13:44-51, 1987
96. Daneman D, Clarson C: Residual beta-cell function in children with type 1 diabetes: measurement and impact on glycemic control. *Clin Invest Med* 10:484-487, 1987
97. Arnold-Larsen S, Madsbad S, Kuhl C: Reproducibility of the glucagon test. *Diabet Med* 4:299-303, 1987
98. Ludvigsson J: Methodological aspects on C-peptide measurements. *Acta Med Scand Suppl* 671:53-59, 1983
99. Madsbad S, Sauerbrey N, Moller-Jensen B, Krarup T, Kuhl C: Outcome of the glucagon test depends upon the prevailing blood glucose concentration in type I (insulin-dependent) diabetic patients. *Acta Med Scand* 222:71-74, 1987
100. Gjessing HJ, Reinholdt B, Faber OK, Pedersen O: The effect of acute hyperglycemia on the plasma C-peptide response to intravenous glucagon or to a mixed meal in insulin-dependent diabetes mellitus. *Acta Endocrinol (Copenh)* 124:556-562, 1991
101. Mandrup-Poulsen T, Molvig J, Andersen HU, Helqvist S, Spinass GA, Munk M: Lack of predictive value of islet cell antibodies, insulin antibodies, and HLA-DR phenotype for remission in cyclosporin-treated IDDM patients: the Canadian-European Randomized Control Trial Group. *Diabetes* 39:204-210, 1990
102. McGregor AM, Petersen MM, McLachlan SM, Rooke P, Smith BR, Hall R: Carbimazole and the autoimmune response in Graves' disease. *N Engl J Med* 303:302-307, 1980
103. Kahaly G, Pitz S, Muller-Forell W, Hommel G: Randomized trial of intravenously immunoglobulins versus prednisolone in Graves' ophthalmopathy. *Clin Exp Immunol* 106:197-202, 1996

104. Couper JJ, Harrison LC, Aldis JE, Colman PG, Honeyman MC, Ferrante A: IgG subclass antibodies to glutamic acid decarboxylase and risk for progression to clinical insulin-dependent diabetes. *Human Immunol* 59:493-499, 1998
105. Bonifacio E, Scirpoli M, Kredel K, Fuchtenbusch M, Ziegler AG: Early autoantibody responses in prediabetes are IgG1 dominated and suggest antigen-specific regulation. *J Immunol* 163:525-532, 1999
106. Harrison LC, Chu XS, DeAizpurua HJ, Graham M, Honeyman MC, Colman PG: Islet-reactive T cells are a marker of pre-clinical insulin-dependent diabetes. *J Clin Invest* 89:1161-1165, 1992
107. Durinovic-Bellò I, Hummel M, Ziegler A: Cellular immune response to diverse islet cell antigens in IDDM. *Diabetes* 45:795-800, 1996
108. Honeyman MC, Brusica V, Stone N, Harrison LC: Neural network-based prediction of candidate T-cell epitopes. *Nature Biotech* 16:966-970, 1998
109. Roep BO, Atkinson MA, van Endert PM, Gottlieb PA, Wilson SB, Sachs JA: Autoreactive T cell responses in insulin-dependent (type 1) diabetes mellitus: report of the First International Workshop for Standardization of T cell Assays. *J Autoimmun* 13:267-282, 1999