

Diabetes-Related Autoantibodies and Gestational Diabetes

ALBERTO DE LEIVA, MD, PHD, MHE, FACE^{1,2,3}
DÍDAC MAURICIO, MD, PHD¹
ROSA CORCOY, MD, PHD^{1,2,3}

Gestational diabetes mellitus (GDM) has long been recognized as a heterogeneous disorder (1,2), with autoimmunity against the β -cell contributing in a small subset of patients (3).

HETEROGENEITY OF AUTOIMMUNE DIABETES: AN OVERVIEW

Autoimmune diabetes is caused by the destruction of β -cells of pancreatic islets by an immune-mediated process, promoted by the interaction of genetic and environmental factors (4). Autoantibodies (AAs) against pancreatic β -cell antigens precede the clinical onset of type 1 diabetes (4). Circulating islet cell antibodies, originally described by indirect immunofluorescence in 1974 (5), have been demonstrated in the great majority of individuals with type 1 diabetes, both at the pre-clinical state and at the onset of clinically overt diabetes, and they persist in the circulation for a long time. Islet cell AAs include autoantibodies to islet cell cytoplasm (islet cell autoantibodies [ICAs]), to native insulin (insulin autoantibodies [IAAs]), to GAD (GADA) (6–8), and to tyrosine phosphatases (insulinoma-associated antigens IA-2A and IA-2B) (9,10).

Age not only modifies the risk of autoimmune diabetes, but also the presence of AAs, the intensity of β -cell destruction, the rate of progression to overt diabetes, and the degree of residual insulin secretion. Approximately 30% of subjects with classic autoimmune diabetes (type 1A di-

abetes) present after age 35 years (11). Childhood autoimmune diabetes is associated with an increased prevalence of alleles DR3, DQB1*0201 and DR4, and DQB1*0302, with the proportion of heterozygotes declining with age at diagnosis (12). Children with the allele HLA DR2, DQB1*0602, almost never develop diabetes, whereas this allele confers a much lower protection for adult-onset autoimmune diabetes (13).

Since the discovery of AAs against islet cell antigens, it has been recognized that a fraction of adults considered to have type 2 diabetes probably have autoimmune diabetes and that the presence of GADAs indicates a strong possibility of requiring insulin treatment earlier. These patients with adult-onset autoimmune diabetes can be initially easily misclassified as having type 2 diabetes. Actually, assessment of diabetes-related autoantibodies (DRAs) might allow them to be classified as having latent autoimmune diabetes in adults (LADA). Characteristically, they display a lower rate of metabolic syndrome than patients with type 2 diabetes (14). The distinction between adult-onset type 1 diabetes and LADA is sometimes difficult, but, characteristically, patients with LADA evolve slowly toward insulin requirement (within 6 years) and older patients with LADA show even a slower progression (15,16).

DRAs AND DIABETIC PREGNANCY

Prevalence and titers

ICAs. The prevalence of cytoplasmic islet cell antibodies in GDM has been reported to range between 0.98 and 14.7% in Caucasians (1,2,3,17–37). Only some authors have studied a control group, with ICAs being reported to be higher in women with GDM (1,24,29,32,36,37), except in studies with low statistical power (26,28). Characteristically, ICA titers are low when compared with subjects with new-onset type 1A diabetes and their first-degree relatives (19,24,26,28,32,36) (Table 1).

Our group has compared ICA titers in 38 ICA⁺ women with GDM and 66 women with new-onset type 1 diabetes and results are displayed in Fig. 1.

IAAs. The presence of IAAs in sera of subjects with type 1 diabetes before initiating insulin treatment was first reported in 1982 (38). Since then, IAAs have been shown in 20–50% of patients with type 1 diabetes of recent diagnosis (39,40). IAA positivity displays a strong negative association with age: IAAs are positive in 90% of children who develop type 1 diabetes before the age of 5 years, but in <40% after the age of 12 years (41). This result is in accordance with the low prevalence of IAAs in women with GDM (0–5.9%), even when addressed just by a low number of reports (3,26,27,28,37), with only one of them reporting a higher prevalence than in the control population (37). When positive, IAA titers are also lower than those of patients with type 1 diabetes and their first-degree relatives (28).

We have investigated the frequency of IAAs in women with GDM using a radio-binding assay. We observed that only 0.98% (2 of 203) of consecutive women at diagnosis of GDM displayed IAAs in their sera before initiation of treatment (registered frequency in control subjects, 0 of 106 individuals; frequency in first-degree relatives, 4.7%). Interestingly, in the subgroup of ICA⁺ women with GDM, the prevalence of IAAs was higher than in ICA⁻ ones (11 versus 0.7%), so that in women with GDM and ICA positivity, the prevalence of IAAs was not different than in first-degree relatives. (Table 2) (3).

From ¹Servei d'Endocrinologia i Nutrició, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; the ²Departament de Medicina, Universitat Autònoma de Barcelona, Barcelona, Spain; and the ³Centro de Investigación Biomédica del Área de Bioingeniería, Biomateriales y Nanotecnología, Instituto de Salud Carlos III, Barcelona, Spain.

Address correspondence and reprint requests to Prof. Dr. Alberto de Leiva, Servei d'Endocrinologia i Nutrició, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Avinguda Sant Antoni M. Claret, 167, 08025, Barcelona, Spain. E-mail: aleiva@santpau.es.

Received for publication 28 March 2006 and accepted in revised form 15 May 2006.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from LifeScan, Inc., a Johnson & Johnson company.

Abbreviations: AA, autoantibody; DRA, diabetes-related autoantibody; GADA, autoantibodies to GAD; IA, insulin antibody; IAA, insulin autoantibody; ICA, islet cell autoantibody; LADA, latent autoimmune diabetes in adults.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

DOI: 10.2337/dc07-s204

© 2007 by the American Diabetes Association.

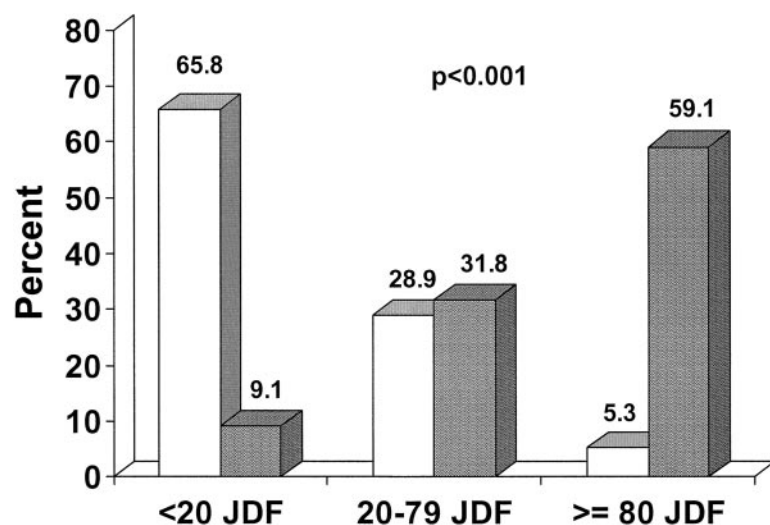


Figure 1—ICA titers: GDM versus type 1 diabetes. □, GDM; ■, type 1 diabetes.

Quite a different issue is the appearance of insulin antibodies (IAs) in women with GDM treated with exogenous insulin. Our group has reported that 44% of women receiving human insulin therapy for GDM develop IAs, which may persist up to 24 months after delivery (42). Similar findings have been described after treatment with lispro (43).

GADAs and IA-2As. GAD is the biosynthesizing enzyme of γ -aminobutyric acid. Pancreatic β -cells express this enzyme, among other cell types. The prevalence of these AAs in subjects with new-onset type 1 diabetes is 60–70% for GADAs, 40% for IA-2As, and 20% for IA-2 β As (44,45). All DRAs could be useful for screening of autoimmune diabetes. Because ICA measurement is time-consuming and semi-quantitative, currently it is usually used for confirmation purposes. The combination of two antibodies offers a good yield with GADAs/IA-2As and GADAs/IAAs being used in adults and children, respectively (46). Our group has recently reported that nonisotopic alternatives for GADA65 and IA-2As are suitable for precise use of estimation of risk prediction and diagnosis of autoimmune diabetes (47).

A few reports have shown that the prevalence of IA-2As in GDM ranges from 0 to 6.2% (28–30,32,34,35,37). Four articles have compared the prevalence of IA-2As in women with GDM with that of a control population, the figures being higher in two articles (29,32) and similar in the other two (28,37). These antibodies are not frequent in this age range (48) and are associated with rapid progression to severe insulinopenia (49). IA-2A titers in

women with GDM are lower than in children with type 1A diabetes (30).

The prevalence of GADAs in GDM has been reported to range from 0 to 10.8% (28–30,32–37,50–56), with an article from India describing a 41% prevalence of GADAs/IA-2As (57). Several reasons may account for these heterogeneous findings. First, each population has a different genetic and environmental background that confers different risks. Second, in each population, different ethnic groups may have different susceptibility for GDM and to β -cell autoimmunity. Third, methodological issues, such as study design or laboratory procedures, may justify these wide differences. As in the case of ICAs, their frequency has been reported to be higher than in the control population in some (29,32,36,37,55) but not all (28,52,53,56) articles, probably because of the low statistical power of the latter. As for other DRAs, titers of GADAs in GDM have also been reported to be lower when compared with type 1A diabetes, autoimmune pre-diabetes, and first-degree relatives (36,58).

A German multicentric study has shown that GADAs in GDM women bound fewer epitopes than GADAs in first-degree relatives (58). Whereas the prevalence and titers of GADAs to the major GAD65 COOH-terminal middle epitope were not significantly different (91 vs. 100%, 88.6 vs. 100%), AAs to epitopes GAD65-midb (middle epitope) and GAD67 were less frequently detected in patients with GDM (57 vs. 90%, 38 vs. 79%). AAs reactive to all epitopes of GAD65 tested in this study were also less frequent in subjects with GDM, in com-

parison to first-degree relatives (65 vs. 23%, $P < 0.01$). The observed reduction of binding to GAD epitopes in women with GDM, in comparison to young first-degree relatives, is also seen in LADA patients (59).

Similarly to LADA, in GDM, the combined presence of various AAs is infrequent (29,30,32,35,37), which is consistent with the concept of a slow progression form of autoimmune diabetes (58).

Transplacental passage of DRAs and related effects

DRAs are transferred to the fetus (42,60–66). They are mainly IgG and are actively transported by the placenta (67,68). Maternal and fetal levels are highly correlated for IAs (42,60,63–65), ICAs (60), GADAs (60,69), and IA-2As (60). This transplacental passage of antibodies lies behind the higher prevalence of DRA in cord blood from infants of diabetic mothers compared with infants of diabetic fathers (66). Postnatal elimination of transplacentally acquired DRAs has been prospectively investigated in infants born to type 1 diabetic mothers, with the mean elimination time being 3.1 months for ICAs and IAs, 4.5 months for GADAs, and 4.3 months for IA-2As (60).

Menon et al. (63) reported that the concentration of animal insulin in cord serum correlated with birth weight, but this observation has not been confirmed by other authors (42,64,65,70,71). The underlying mechanism would be an IA-facilitated maternal insulin transfer and, as a consequence, an enhancement of fetal hyperinsulinism and related morbidity (72). Maternal insulin-IA complexes have been associated with toxemia (18,73,74), HELLP syndrome (75), hypoglycemia (18,76), respiratory distress (18), and high hematocrit in the newborn (76). Nevertheless, other investigators have not observed a relationship between maternal levels of IAs and cord blood levels of insulin (71), 32–33 split proinsulin (71), or C-peptide (64). In our experience, fetal outcomes were not influenced by the presence or titers of IAs, IAAs, or ICAs (77).

In the nonobese diabetic mouse, the transplacental passage of maternal AAs increases the risk of experimental autoimmune diabetes in the offspring (78), whereas the transmission of AAs against activated T-cells is protective (79). Interestingly, in infants of diabetic mothers, the presence of GADAs and IA-2As, but

Table 1—DRAs in women with GDM

Author	n	ICA prevalence (%)	IAA prevalence (%)	GADA prevalence (%)	I-A2A prevalence (%)
Steel et al. (17)	50	10			
Ginsberg-Fellner et al. (20)*	88	35			
Fallucca et al. (18)	39	5			
Freinkel et al. (1)	160	7.5†			
Stowers et al. (19)‡	72	12.5			
Catalano et al. (21)‡	187	1.6			
Bell et al. (22)	181	2.8			
Stangerberg et al. (23)	55	1.8			
Mauricio et al. (24)	307	12.4†			
Ziegler et al. (25)	55	11			
Damm et al. (26)	139	2.9§	0§		
Tuomilehto et al. (50)	112			5.0	
Beischer et al. (51)‡	734			1.8	
Mauricio et al. (3)	203		1		
Petersen et al. (52)	139			2.2§	
Lapolla et al. (27)	68	2.9	1.5		
Dozio et al. (28)	145	10§	3.0§	0§	0§
Fuchtenbusch et al. (29)	437	8.5†		9.5†	6.2†
Fallucca et al. (53)	83			3.6§	
Whittingham et al. (30)	98	3		4	1
Panczel et al. (31)‡	68	14.7			
Kinalski et al. (32)‡	156	5.1†		7.0†	3.2†
Mitchell et al. (54)	100			6†	
Bartha et al. (33)‡	102	0.98		10.8	
Kousta et al. (55)‡	321			4.0	
Weng et al. (56)‡	66			4.5§	
Balaji et al. (57)	86			41 GADA/IA-2†	
Lapolla et al. (34)	70	2.8		1.4	0
Albareda et al. (35)	535	14		1.5	0.2
Bo et al. (36)	123	6.5†		4.1†	
Järvelä et al. (37)	435	12.5†	5.9†	4.7†	1.0§

Adapted from Lauenborg et al. (84). For groups with several articles on the subject, the first article reporting the prevalence of each autoantibody is included. *The method was later shown to produce false-positive results. † $P < 0.05$ vs. the control population. ‡Measurements were performed at different times after delivery; §NS vs. the control population. ¶Women had both GDM and a positive family history of diabetes.

not of IAs, in cord blood samples is associated with a lower risk of developing DRAs and type 1 diabetes (80). However, when present in infants of diabetic fathers, DRAs are not protective, which seems to imply an active fetus self-immune response in this case (80).

DRAs AND THE RISK OF MATERNAL GLUCOSE INTOLERANCE/DIABETES— A major issue regarding the presence of DRAs in women with GDM is the potentially increased risk for the development of diabetes either at short term after delivery or at longer follow-up. The majority of reports have agreed that positivity for DRA during pregnancy increases the maternal risk of glucose intolerance/diabetes (Table 3).

In the first investigation on the prevalence of ICAs in GDM, three of five ICA⁺ gestational diabetic women developed classic type 1A diabetes shortly after pregnancy (17). Additional studies have confirmed an increased risk of diabetes (24) or glucose intolerance (21) in women positive for ICAs. The association has also been extended to other DRAs: positivity for either ICA/IA-2A or GADAs increases the risk of type 1 diabetes 2 years after delivery, with the risk increasing with the number of positive antibodies (29). There are also articles reporting a lack of association of ICAs (23) or GADAs (56) with abnormal glucose tolerance at short term after delivery that could be attributable to a low statistical power of the studies. Nevertheless, it is important to highlight that only two of these articles performed sta-

tistical adjustment for other predictors (23,29).

In addition to ICAs being predictive of diabetes at the first assessment after delivery (24), our team reported an impairment of the acute insulin response to intravenous glucose in women with GDM with positivity for ICAs and normal glucose tolerance after delivery (81). The response was superimposable to that of ICA⁺ first-degree relatives. Interestingly, a Finnish study on first-degree relatives of LADA patients demonstrated similar metabolic features that were described by us in women with GDM and positivity for ICAs. These individuals (family members of LADA patients) exhibited decreased insulin secretion, associated with an increased prevalence of risk genotypes (82).

At longer follow-up, an increased risk of type 1 diabetes has been demonstrated for ICAs (20,26,31,37,52), GADAs (37,49,52), and positivity for one or more islet cell antibodies (52,83–85), with the risk increasing with the number of antibodies (37), but not for IA-2 (29,37). Even for ICAs and GADAs, not all articles find a positive association between DRA positivity and diabetes at follow-up, which in some cases (19,34), but not others (35,55), can be attributed to a low statistical power. For example, in our population, despite the aforementioned association of ICA positivity with postpartum abnormal glucose tolerance, DRA positivity (ICA/GADA/IA-2, alone or in any combination) was not predictive of diabetes at mid-term follow-up (35). As in the case of short-term follow-up, only some studies have adjusted for other predictors (35,37,83).

AUTOIMMUNE GDM: A DISTINCT PRE-DIABETIC STAGE

— A recent publication investigated the differences in clinical character-

Table 2—Prevalence of IAAs in women with GDM according to ICA status, as compared with first-degree relatives of subjects with type 1 diabetes

	n	Prevalence
GDM		
ICA ⁺	27	11%
ICA ⁻	135	0.7%
FDR		
ICA ⁺	34	10%
ICA ⁻	23	4%

Adapted from Mauricio et al. (3).

Table 3—Abnormal glucose tolerance at follow-up in women with GDM and DRAs

Author	Follow-up	ICAs	IAAs	GADAs	IA-2As	Several DRAs
Steel et al. (17)	1 year	Predictive of type 1 diabetes				
Ginsberg-Fellner et al. (20)	Years	Predictive of type 1 diabetes				
Stowers et al. (19)	Up to 22 years	Not predictive of the final state of glucose tolerance				
Catalano et al. (21)	Up to 4 years	Predictive of IGT				
Stangerberg et al. (23)	2–4 months	Not predictive of abnormal OGTT*				
Mauricio et al. (24)	Months	Predictive of diabetes				
Damm et al. (26)	Up to 11 years	Predictive of type 1 diabetes				
Beischer et al. (51)	Years					
Petersen et al. (52)	Up to 11 years	Predictive of type 1 diabetes		GADAs at follow-up associated with type 1 and type 2 diabetes Predictive of type 1 diabetes		DRA positivity predictive of type 1 diabetes
Fuchtenbusch et al. (29)	Up to 5 years	Predictive of type 1 diabetes*		Predictive of type 1 diabetes*	Not predictive of type 1 diabetes*	Risk of type 1 diabetes increases with the number of DRAs*
Panczel et al. (31)	Up to 14 years					
Kousta et al. (55)	Up to 45 months	Predictive of type 1 diabetes		GADAs at follow-up not associated with different FBG or HOMA estimations of insulin secretion and sensitivity No association with diabetes/IGT		
Weng et al. (56)	1 year					
Lapolla et al. (34)	5 years					DRA positivity, borderline association to type 1 diabetes
Albareda et al. (35)	Up to 11 years					DRA positivity not predictive of diabetes, type 1 diabetes, or type 2 diabetes*
Järvelä et al. (37)	Up to 7 years	Predictive of type 1 diabetes*		Predictive of type 1 diabetes*	Not predictive of type 1 diabetes*	Number of DRAs predictive of type 1 diabetes*
Löbner et al. (83)	Up to 11 years					GAD and/or IA-2 positivity predictive of diabetes*

For groups with several articles on the subject, only articles providing new information on the predictive ability of DRAs are included. * Adjusted for other predictors. FBG, fasting blood glucose; HOMA, homeostasis model assessment; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test.

istics of women with GDM, whether associated with islet cell autoimmunity or not. A total of 207 women composed the total group of investigated subjects; 12.5% were carrying AAs in their sera, either ICAs and/or GADAs, usually at low titers. GDM women with autoimmune markers presented lower a priori risk for the development of GDM (were younger, had lower prepregnancy BMI, lower prevalence of diabetes in first-degree relatives, lower waist circumference, lower fasting plasma insulin, and lower weight increase during pregnancy). The rate of insulin treatment during pregnancy was significantly higher in the group positive for AAs (36). These observations led the authors to the conclusion that women with autoimmune GDM displayed fewer features of insulin resistance, required more frequent insulin therapy than negative women, and presumably had presymptomatic type 1 diabetes. Nevertheless, other authors have reported that women with GDM with or without GADA positivity at follow-up display similar clinical characteristics with the exception of BMI (55).

Freinkel et al. (2) foresaw the evolution of what may be defined as autoimmune GDM, when they wrote in 1987, that GDM entails genotypic and phenotypic diversity and may include patients with slowly evolving type 1 diabetes. Seventeen years after such publication, and considering today's new information, we believe that autoimmune GDM is a heterogeneous condition, covering for ~10% of all Caucasian women diagnosed with GDM. This condition may display the various types of expression of the immune reactivity against the β -cell. With these considerations, we propose that autoimmune GDM be regarded as a distinct clinical entity. This proposal does not only make reference to a special subtype of GDM for academic or classification purposes; it also addresses a peculiar and complex pre-diabetic status, susceptible of future new strategies for diabetes prevention.

Acknowledgments—We thank all participants of the Spanish Pre-Diabetes Study Group; Professor Lluís Cabero and Dr. Joan Adelantado, from the Department of Obstetrics and Gynecology, Universitat Autònoma de Barcelona; Drs. Mercedes Albareda, Montserrat Balsells, Jaume Binimelis, Mercedes Codina, Gemma Ginovart, Eugenia Mato, Josefa Morales, Xavier Palomer, Sandra Piquer, and Manel Puig-Domingo, for their collaboration

in different phases of the research team activities and related publications; Eulalia Brugués, MSc (Pharmacy), for efficient help in the preparation of the manuscript; all registered nurses of the Departments of Endocrinology and Diabetes, Obstetrics and Gynecology, and Pediatrics of the Hospital de la Santa Creu i Sant Pau for the excellent services offered to all women and their newborns attended at the institution, and related to this publication.

References

- Freinkel N, Metzger BE, Phelps RL, Dooley SL, Ogata ES, Radvany RM, Belton A: Gestational diabetes mellitus: heterogeneity of maternal age, weight, insulin secretion, HLA antigens, and islet cell antibodies and the impact of maternal metabolism on pancreatic B-cell and somatic development in the offspring. *Diabetes* 34 (Suppl. 2):1–7, 1985
- Freinkel N, Metzger BE, Phelps RL, Simpson JL, Martin AO, Radvany R, Ober C, Dooley SL, Depp RO, Belton A: Gestational diabetes mellitus: a syndrome with phenotypic and genotypic heterogeneity. *Horm Metab Res* 18:427–430, 1986
- Mauricio D, Balsells M, Morales J, Corcoy R, Puig-Domingo M, de Leiva A: Islet cell autoimmunity in women with gestational diabetes and risk of progression to insulin-dependent diabetes mellitus. *Diabete Metab Rev* 12:275–285, 1996
- Eisenbarth GS: Type 1 diabetes mellitus: a chronic autoimmune disease. *N Engl J Med* 314:1360–1368, 1986
- Bottazzo GF, Florin-Christensen A, Doniach D: Islet cell antibodies in diabetes mellitus with polyendocrine autoimmune deficiencies. *Lancet* 2:1279–1282, 1974
- Baekkeskov S, Aanstoot HJ, Christgau S, Rietz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P: Identification of the 64 autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 347:151–156, 1990
- Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ: Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. *J Clin Invest* 89:283–292, 1992
- Atkinson MA, Maclaren NK: Islet cell autoantigens in insulin dependent diabetes mellitus. *J Clin Invest* 92:1608–1616, 1993
- Lan MS, Wassefall C, Maclaren NK, Notkins AL: IA-2, a transmembrane protein of the protein tyrosine phosphatase family, as an autoantigen in insulin dependent diabetes mellitus. *Proc Natl Acad Sci U S A* 93:6367–6370, 1996
- Lu J, Li Q, Xie H, Chen ZJ, Borovitskaya AE, Maclaren NK, Notkins AL, Lan MS: Identification of a second transmembrane protein tyrosine phosphatase, IA-2beta, as an autoantigen in insulin dependent diabetes mellitus: precursor of the 37-kDa tryptic fragment. *Proc Natl Acad Sci U S A* 93:2307–2311, 1996
- Leslie RD, Delli Castelli M: Age-dependent influences on the origins of autoimmune diabetes: evidence and implications. *Diabetes* 53:3033–3040, 2004
- Horton V, Stratton I, Bottazzo GF, Shattock M, Mackay I, Zimmet P, Manley S, Holman R, Turner R, the UK Prospective Diabetes Study (UKPDS) Group: Genetic heterogeneity of autoimmune diabetes: age at presentation in adults is influenced by HLA DRB1 and DQB1 genotypes (UKPDS 43). *Diabetologia* 42:608–616, 1999
- Sabbah E, Savola K, Ebeling T, Kulmala P, Vahasalo P, Ilonen J, Salmela PI, Knip M: Genetic, autoimmune, and clinical characteristics of childhood and adult-onset type 1 diabetes. *Diabetes Care* 23:1326–1332, 2000
- Pozzilli P, Di Mario U: Autoimmune diabetes not requiring insulin at diagnosis (latent autoimmune diabetes of the adult): definition, characterization, and potential prevention. *Diabetes Care* 24:1460–1467, 2001
- Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF, Holman R: UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes: UK Prospective Diabetes Study Group. *Lancet* 350:1288–1293, 1997
- Zimmet PZ, Tuomi T, Mackay IR, Rowley MJ, Knowles W, Cohen M, Lang DA: The role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. *Diabet Med* 11:299–303, 1994
- Steel JM, Irvine WJ, Clarke BF: The significance of pancreatic islet cell antibody and abnormal glucose tolerance during pregnancy. *J Clin Lab Immunol* 4:83–85, 1980
- Fallucca F, Di Mario U, Gargiulo P, Iavicoli M, Galfo C, Contreas G, Pachi' A, Andreani D: Humoral immunity in diabetic pregnancy: interrelationships with maternal/neonatal complications and maternal metabolic control. *Diabete Metab* 11:387–395, 1985
- Stowers JM, Sutherland HW, Kerridge DF: Long-range implications for the mother: the Aberdeen experience. *Diabetes* 34 (Suppl. 2):106–110, 1985
- Ginsberg-Fellner F, Mark EM, Nechemias C, Hausknecht RU, Rubinstein P, Doberstein NJ, Notkins AL: Islet cell antibodies in gestational diabetics. *Lancet* 2:362–363, 1980
- Catalano PM, Tyzbit ED, Sims EA: Incidence and significance of islet cell antibodies in women with previous gestational diabetes. *Diabetes Care* 13:478–

- 482, 1990
22. Bell DS, Barger BO, Go RC, Goldenberg RL, Perkins LL, Vanichanan CJ, Roseman J, Acton RT: Risk factors for gestational diabetes in black population. *Diabetes Care* 13:1196–1201, 1990
 23. Stangerberg M, Agarwal N, Rahman F, Sheth K, Al Sedeiry S, De Vol E: Frequency of HLA genes and islet cell antibodies (ICA) and result of postpartum oral glucose tolerance tests (OGTT) in Saudi Arabian women with abnormal OGTT during pregnancy. *Diabetes Res* 14: 9–13, 1990
 24. Mauricio D, Corcoy R, Codina M, Balsells M, Puig-Domingo M, Pou JM, de Leiva A: Islet cell antibodies identify a subset of gestational diabetic women with higher risk of developing diabetes mellitus shortly after pregnancy. *Diab Nutr Metab* 5:1–5, 1992
 25. Ziegler AG, Hillebrand B, Rabl W, Mayrhofer M, Hummel M, Mollenhouer U, Vordemann J, Lenz A, Standl E: On the appearance of islet associated autoimmunity in offspring of diabetic mothers: a prospective study from birth. *Diabetologia* 36:402–408, 1993
 26. Damm P, Kuhl C, Buschard K, Kakobsen BK, Svedjaard A, Sodoyez-Goffaux F, Shattock M, Bottazzo GF, Molsted-Pedersen L: Prevalence and predictive value of islet cell antibodies and insulin autoantibodies in women with gestational diabetes. *Diabet Med* 11:558–563, 1994
 27. Lapolla A, Betterle C, Sanzari M, Zanchetta R, Pfeifer E, Businaro A, Fagiolo U, Plebani M, Marini S, Photiou E, Fedele D: An immunological and genetic study of patients with gestational diabetes mellitus. *Acta Diabetol* 33:139–144, 1996
 28. Dozio N, Beretta A, Belloni C, Castiglioni M, Rosa S, Bosi E, Bonifacio E: Low prevalence of islet autoantibodies in patients with gestational diabetes mellitus. *Diabetes Care* 20:81–83, 1997
 29. Fuchtenbusch M, Ferber K, Standl E, Ziegler AG: Prediction of type 1 diabetes postpartum in patients with gestational diabetes mellitus by combined islet cell autoantibody screening: a prospective multicenter study. *Diabetes* 46:1459–1467, 1997
 30. Whittingham S, Byron SL, Tuomilehto J, Zimmet PZ, Myers MA, Vidgren G, Rowley MJ, Feeney SJ, Koskela P, Tuomilehto-Wolf E, Mackay IR: Autoantibodies associated with presymptomatic insulin-dependent diabetes mellitus in women. *Diabet Med* 14:678–685, 1997
 31. Panczel P, Kulkey O, Luczay A, Bornemisza B, Illyes G, Halmos T, Baranyi E, Blatniczky L, Meszaros J, Kerenyi Z, Gero L, Tamas G, Hosszufalusi N, Horvath L, Madacsy L, Romics L: Detection of antibodies against pancreatic islet cells in clinical practice. *Orv Hetil* 140:2695–2701, 1999
 32. Kinalski M, Kretowski A, Telejko B, Kowalska I, Bingley P, Kinalska I: Prevalence of ICA antibodies, anti-GAD and anti-IA2 in women with gestational diabetes treated with diet. *Przegl Lek* 56:342–346, 1999
 33. Bartha JL, Martínez del Fresno P, Comino-Delgado R: Postpartum metabolism and autoantibody markers in women with gestational diabetes mellitus diagnosed in early pregnancy. *Am J Obstet Gynecol* 184:965–970, 2001
 34. Lapolla A, Fedele D, Pedini B, Dal Fra MG, Sanzari M, Masin M, Zanchetta R, Betterle C: Low frequency of autoantibodies to islet cell, glutamic acid decarboxylase and second-islet antigen in patients with gestational diabetes mellitus: a follow-up study. *Ann N Y Acad Sci* 958:263–266, 2002
 35. Albareda M, Caballero A, Badell G, Piquer S, Ortiz A, de Leiva A, Corcoy R: Diabetes and abnormal glucose tolerance in women with previous gestational diabetes. *Diabetes Care* 26:1199–1205, 2003
 36. Bo S, Menato G, Pinach S, Signorile A, Bardelli C, Lezo A, Marchisio B, Gentile L, Cassader M, Massobrio M, Pagano G: Clinical characteristics and outcome of pregnancy in women with gestational hyperglycemia with and without antibodies to beta-cell antigens. *Diabet Med* 20:64–68, 2003
 37. Järvelä I, Juutinen J, Koskela P, Hartikainen AL, Kulmala P, Knip M, Tapanaianen JS: Gestational diabetes identifies women at risk for permanent type 1 and type 2 diabetes in fertile age: predictive role of autoantibodies. *Diabetes Care* 29: 607–612, 2006
 38. Palmer JP, Asplin CH, Clemons P: Insulin antibodies in insulin dependent diabetics before insulin treatment. *Science* 222: 1337–1339, 1982
 39. Karjalainen J, Salmela P, Ilonen J, Surcel HM, Knip M: A comparison of childhood and adult type 1 diabetes mellitus. *N Engl J Med* 320:881–886, 1989
 40. Srikantha S, Ricker AT, McCulloch DK, Soeldner JS, Eisenbarth GS, Palmer JP: Autoimmunity to insulin, beta-cell dysfunction and development of insulin-dependent diabetes mellitus. *Diabetes* 36: 139–142, 1986
 41. Naserke HE, Dozio N, Ziegler AG, Bonifacio E: Comparison of a novel microassay for insulin autoantibodies with the conventional radiobinding assay. *Diabetologia* 41:681–683, 1998
 42. Balsells M, Corcoy R, Mauricio D, Morales J, García-Patterson A, Carreras G, Puig-Domingo M, de Leiva A: Insulin antibody response to a short course of human insulin therapy in women with gestational diabetes. *Diabetes Care* 20:1172–1175, 1997
 43. Jovanovic L, Ilic S, Pettitt DJ, Hugo K, Gutierrez M, Bowsher RR, Bastyr EJ: Metabolic and immunologic effects of insulin lispro in gestational diabetes. *Diabetes Care* 22:1422–1427, 1999
 44. Seissler J, Scherbaum WA: Autoimmune diagnostics in diabetes mellitus. *Clin Chem Lab Med* 44:133–137, 2006
 45. Leslie RDG, Atkinson MA, Notkins AL: Autoantigens IA₂ and GAD in type 1 (insulin-dependent) diabetes. *Diabetologia* 42:3–14, 1999
 46. Bingley PJ, Bonifacio E, Ziegler AG, Schatz DA, Atkinson MA, Eisenbarth GS, Immunology of Diabetes Society: Proposed guidelines on screening for risk of type 1 diabetes. *Diabetes Care* 24:398, 2001
 47. Palomer X, Mauricio D, Rodriguez-Espinosa J, Zapico E, Mayoral C, Gonzalez-Sastre F, de Leiva A, Blanco-Vaca F: Evaluation of two non-isotopic immunoassays for determination of glutamic acid decarboxylase and tyrosine phosphatase autoantibodies in serum. *Clin Chem* 50: 1378–1382, 2004
 48. Lohmann T, Sessler J, Verloren HJ, Schroder S, Rotger J, Dahn K, Morgenthaler N, Scherbaum WA: Distinct genetic and immunological features in patients with onset of IDDM before and after age 40. *Diabetes Care* 20:524–529, 1997
 49. Mayrhofer M, Rabin DU, Messenger L, Standl E, Ziegler AG: Value of ICA512 antibodies for prediction and diagnosis of type 1 diabetes. *Exp Clin Endocrinol Diabetes* 104:228–234, 1996
 50. Tuomilehto J, Zimmet P, Mackay IR, Koskela P, Vidgren G, Toivanen L, Tuomilehto-Wolf E, Kohtamaki K, Stengard J, Rowley MJ: Antibodies to glutamic acid decarboxylase as predictors of insulin-dependent diabetes mellitus before clinical onset of disease. *Lancet* 343:1383–1385, 1994
 51. Beischer NA, Wein P, Sheedy MT, Mackay IR, Rowley MJ, Zimmet P: Prevalence of antibodies to glutamic acid decarboxylase in women who have had gestational diabetes. *Am J Obstet Gynecol* 173:1563–1569, 1995
 52. Petersen JS, Dyrberg T, Damm P, Kuhl C, Molsted-Petersen L, Buschard K: GAD65 autoantibodies in women with gestational or insulin dependent diabetes mellitus diagnosed during pregnancy. *Diabetologia* 39:1329–1333, 1996
 53. Fallucca F, Tiberti C, Torresi P, Cardellini G, Sciuolo E, D'Aliberti T, Napoli A, Di Mario U: Autoimmune markers of diabetes in diabetic pregnancy. *Ann Ist Super Sanita* 33:425–428, 1997
 54. Mitchell ML, Hermos RJ, Larson CA, Palomaki GE, Haddow JE: Prevalence of GAD autoantibodies in women with gestational diabetes mellitus. *Diabetes Care* 23:1705–1706, 2000
 55. Kousta E, Lawrence NJ, Anyaoku V, Johnston DG, McCarthy MI: Prevalence and features of pancreatic islet cell auto-

- immunity in women with gestational diabetes from different ethnic groups. *BJOG* 108:716–720, 2001
56. Weng J, Ekelund M, Lehto M, Li H, Ekberg G, Frid A, Aberg A, Groop LC, Berntorp K: Screening for MODY mutations, GAD antibodies, and type 1-diabetes associated HLA genotypes in women with gestational diabetes mellitus. *Diabetes Care* 25:68–71, 2002
 57. Balaji M, Shatauvare-Brameus A, Valaji V, Seshiah V, Sanjeevi CB: Women diagnosed with gestational diabetes mellitus do not carry antibodies against minor cell antigens. *Ann N Y Acad Sci* 958:281–284, 2002
 58. Fuchtenbusch M, Bonifacio E, Lampasona V, Knopff, Ziegler AG: Immune responses to glutamic acid decarboxylase and insulin in patients with gestational diabetes. *Clin Exp Immunol* 135:318–321, 2004
 59. Hawa MI, Fava D, Medici F, Deng YJ, Notkins AL, De Mattia G, Leslie RD: Antibodies to IA-2 and GAD65 in type 1 and type 2 diabetes: isotype restriction and polyclonality. *Diabetes Care* 23:228–233, 2000
 60. Hämäläinen AM, Ronkainen MS, Akerman HK, Knip M: Postnatal elimination of transplacentally acquired disease-associated antibodies in infants born to families with type 1 diabetes: the Finnish TRIGR Study Group: Trial to reduce IDDM in the Genetically at Risk. *J Clin Endocrinol Metab* 85:4249–4253, 2000
 61. Spellacy WN, Goetz FC: Insulin antibodies in pregnancy. *Lancet* 2:222–224, 1963
 62. Gamlen TR, Aynsley-Green A, Irvine WJ, McCallum CJ: Immunological studies in the neonate of a mother with Addison's disease and diabetes mellitus. *Clin Exp Immunol* 28:192–195, 1977
 63. Menon RK, Cohen RM, Sperling MA, Cutfield WS, Mimouni F, Khouri JC: Transplacental passage of insulin in pregnant women with insulin-dependent diabetes mellitus: its role in fetal macrosomia. *N Engl J Med* 323:309–315, 1990
 64. Mylvaganam R, Stowers JM, Steel JM, Wallace J, MacHendry JC, Wright AD: Insulin immunogenicity in pregnancy: maternal and fetal studies. *Diabetologia* 24: 19–25, 1983
 65. Weiss PA, Kainer F, Purstner P, Zehetleitner G, Huttner U, Haas J: Anti-insulin antibodies and birth weight in pregnancies complicated by diabetes. *Early Hum Dev* 53:145–154, 1998
 66. Roll U, Christie MR, Fuchtenbusch M, Payton MA, Hwkes CJ, Ziegler AG: Perinatal autoimmunity in offspring of diabetic parents: the German Multicenter BABY-DIAB study: detection of humoral immune responses of islet antigens in early childhood. *Diabetes* 45:967–973, 1996
 67. Omar MA, Srikanta S, Eisenbarth GS: Human islet cell antibodies: immunoglobulin class and subclass distribution defined by monoclonal antibodies. *Diabetes Res* 4:155–157, 1987
 68. Pitcher-Wilmott RW, Hindocha P, Wood CB: The placental transfer of IgG subclasses in human pregnancy. *Clin Exp Immunol* 41:303–308, 1980
 69. Lindberg B, Ivarsson SA, Landin-Olsson M, Sundkvist G, Svanberg L, Lenmark A: Islet autoantibodies in cord blood from children who developed type 1 (insulin dependent) diabetes mellitus before 15 years of age. *Diabetologia* 42:181–187, 1999
 70. Schwartz R, Gruppuso PA, Petzold K, Brambilla D, Hiilesmaa V, Teramo KA: Hyperinsulinemia and macrosomia in the fetus of the diabetic mother. *Diabetes Care* 17:640–648, 1994
 71. Lindsay RS, Ziegler AG, Hamilton BA, Calder AA, Johnstone FD, Walker JD: Scottish Multicentre Study of Diabetes and Pregnancy: Type 1 diabetes-related antibodies in the fetal circulation: prevalence and influence on cord insulin and birth weight in offspring of mothers with type 1 diabetes. *J Clin Endocrinol Metab* 89:3436–3439, 2004
 72. Bauman WA, Yalow RS: Transplacental passage of insulin complexed to antibody. *Proc Natl Acad Sci U S A* 78:4588–4590, 1981
 73. Tamas G Jr, Bekefi D, Gaal O: Insulin antibodies in diabetic pregnancy. *Lancet* 1:521, 1975
 74. Di Mario U, Falluca F, Gargiulo P, Tiberti C, Scardellato A, Arduini P: Insulin-anti-insulin complexes in diabetic women and their neonates. *Diabetologia* 27 (Suppl.): 83–86, 1984
 75. Weitgasser R, Spitzer D, Kartnig I, Zajc M, Staudach A, Sandhofer F: Association of HELLP syndrome with autoimmune antibodies and glucose intolerance. *Diabetes Care* 23:786–790, 2000
 76. Murata K, Toyoda N, Sugiyama Y: The effects of insulin antibodies during diabetic pregnancy on newborn infants. *Asia Oceania J Obstet Gynaecol* 16:115–122, 1990
 77. Balsells M, Corcoy R, Garcia-Patterson A, Morales J, Puig M, de Leiva A: Insulin antibodies and materno-fetal morbidity in gestational diabetic women. *Diabetologia* 37 (Suppl. 1):172A, 1994
 78. Greeley SA, Katsumata M, Yu L, Eisenbarth GS, Moore DJ, Goodarzi H, Barker CF, Naji A, Noorchashm H: Elimination of maternally transmitted autoantibodies prevents diabetes in non-obese diabetic mice. *Nat Med* 8:399–402, 2002
 79. McKeever U, Khandekar S, Newcomb J, Naylor J, Gregory P, Brauer P, Jesson M, Bettencourt B, Burke E, Alderson A, Banerji J, Haskins K, Jones B: Immunization with soluble BDC 2.5 T cell receptor-immunoglobulin chimeric protein: antibody specificity and protection of non obese diabetic mice against adoptive transfer of diabetes by maternal immunization. *J Exp Med* 184:1755–1768, 1996
 80. Koczwara K, Bonifacio E, Ziegler AG: Transmission of maternal islet antibodies and risk of autoimmune diabetes in offspring of mothers with type 1 diabetes. *Diabetes* 53:1–4, 2004
 81. Mauricio D, Corcoy R, Codina M, Morales J, Balsells M, de Leiva A: Islet cell antibodies and beta cell function in gestational diabetic women: comparison to first-degree relatives of type 1 (insulin-dependent) diabetic subjects. *Diabet Med* 12:1009–1014, 1995
 82. Vauhkonen I, Niskanen L, Knip M, Ilonen J, Vanninen E, Kainulainen S, Uusitupa M, Laakso M: Impaired insulin secretion in non-diabetic offspring of probands with latent autoimmune diabetes in adults. *Diabetologia* 43:69–78, 2000
 83. Löbner K, Knopff A, Baumgarten A, Mollenhauer U, Marienfeld S, Garrido-Franco M, Bonifazio E, Ziegler AG: Predictors of postpartum diabetes in women with gestational diabetes mellitus. *Diabetes* 55:792–797, 2006
 84. Lauenborg J, Hansen T, Jensen DM, Vestergaard H, Molsted-Pedersen L, Hornnes P, Loch H, Pedersen O, Damm P: Increasing incidence of diabetes after gestational diabetes: a long-term follow-up in a Danish population. *Diabetes Care* 27: 1194–1199, 2004
 85. Corcoy R, Mauricio D, de Leiva A: Diabetes-related antibodies and pregnancy. In *Diabetology of Pregnancy: Frontiers in Diabetes*. Vol. 17. Djelmis J, Desoye G, Ivanisevic M, Eds. Basel, Karger, 2005, p. 195–205