Disease-Associated Antibodies in Offspring of Mothers with IDDM

Anu Martikainen, Tero Saukkonen, Petri K. Kulmala, Helena Reijonen, Jorma Ilonen, Kari Teramo, Pentti Koskela, Mikael Knip, and Hans K. Åkerblom

We studied 20 infants of mothers with IDDM participating in a pilot study for a dietary intervention trial, testing the hypothesis that avoidance of cow's milk proteins early in life will reduce the risk of subsequent IDDM. The aim was to evaluate the elimination of IDDM-associated antibodies from the peripheral circulation of the infants, the possible emergence of autoantibodies indicating β-cell destruction, and the influence of the dietary intervention and genetic disease susceptibility on the development of these autoantibodies. Transplacentally transferred islet cell antibodies (ICAs) and antibodies to the 65-kDa isoform of glutamic acid decarboxylase (GAD65As) disappeared from the peripheral circulation of most infants over the first few months of life and in all infants before the age of 9 months. Insulin antibodies were eliminated before the same age in all cases but one. The higher the initial antibody level was, the longer the time required for elimination. Four infants tested positive for insulin autoantibodies (IAAs) on at least one occasion during the first year of life, and 5 out of 16 unaffected subjects (31%) had IAAs at the age of 2 years. One infant became positive for IAA before the age of 6 months, with increasing levels later, seroconverted to positivity for ICAs and GAD65As between 6 and 9 months and presented with clinical IDDM at the age of 14 months. He had the HLA DQB1*0302/x genotype, which predisposes carriers to IDDM, and had been given the casein hydrolysate formula as supplementary milk. There were no significant differences in the levels of various autoantibodies between two groups of subjects defined either on the type of dietary intervention or the degree of genetic susceptibility. The findings indicate that transplacentally transferred antibodies related to IDDM are usually eliminated from the peripheral circulation of infants before 9 months of age and that IDDM-associated autoantibodies may emerge before the age of 6 months. Our results also illustrate that avoidance of cow's milk proteins over the first 9 months of life does not provide total protection against IDDM. *Diabetes* 45:1706–1710, 1996

From the Department of Pediatrics (A.M., P.K.K., M.K.), University of Oulu, Oulu; the National Public Health Institute (P.K.), Oulu; I and II Departments of Pediatrics (T.S., H.K.Å.), the Children's Hospital, and the Department of Obstetrics and Gynecology (K.T.), University of Helsinki, Helsinki; and the Turku Immunology Centre and Department of Virology (H.R., J.I.), University of Turku, Turku, Finland.

Address correspondence and reprint requests to Dr. Mikael Knip, Department of Pediatrics, University of Oulu, FIN-90220 Oulu, Finland.

Received for publication 5 March 1996 and accepted in revised form 11 July 1996.

GAD65A, antibody to the 65-kDa isoform of glutamic acid decarbosylase; IA, insulin antibody; IAA, insulin autoantibody; ICA, islet cell antibody; JDF U, Juvenile Diabetes Foundation units; RU, relative units.

The offspring of mothers with IDDM have an approximately five to ten times higher risk of contracting IDDM before the age of 20 years than the general population. The risk is close to 2%, which is about one-third of that for the offspring of fathers with IDDM (6%) (1–3). The majority of insulin-treated diabetic mothers have insulin antibodies (IAs) in response to their exogenous insulin therapy, and though biologically active free insulin does not cross the placenta, IA and insulin-antibody complexes do so with passive transfer (4). Newborn infants of mothers with IDDM may have islet cell antibodies (ICAs) in their circulation as a consequence of transplacental transfer from the maternal circulation (5), and it is conceivable that other IDDM-related antibodies, such as those to the 65-kDa isoform of glutamic acid carboxylase (GAD65As), are also transferred across the placenta, since the majority of these are IgG class antibodies (6).

The genetics of IDDM is a complex interplay of several determinants, the strongest susceptibility being associated with HLA-DQ alleles (7). The alleles significantly associated with disease susceptibility and protection can be defined in the Finnish population by genotyping for four DQB1-specific oligonucleotide probes (8,9). However, only a minority of those with genetic susceptibility to IDDM progress to the clinical disease, which implies that environmental factors triggering and/or potentiating autoimmune β-cell destruction are essential for the development of IDDM. The major environmental candidates are viral infections, some dietary factors, toxins, and stressful life events (10). Initiation of the autoimmune process may precede the clinical onset by several years (11,12). Epidemiological data suggest that a short duration of breast-feeding or early introduction of cow's milk in infancy increases the risk of developing IDDM later (13,14).

The present work is aimed at evaluating the postnatal elimination of transplacentally transferred antibodies associated with IDDM and the emergence of autoantibodies indicating β-cell damage in infants of insulin-treated diabetic mothers. An additional objective was to explore the possible influence of early feeding and genetic susceptibility on the development of IDDM-related autoantibodies.

RESEARCH DESIGN AND METHODS

The population comprised 20 newborn infants of mothers with IDDM recruited for the first pilot study in a dietary intervention trial aimed at evaluating the possible effect of the elimination of cow's milk proteins in early

infancy with the subsequent risk of manifesting IDDM. The infants were born between August 1992 and March 1993 at the Department of Obstetrics and Gynecology, University of Helsinki. In terms of the type of diabetes, classified according to White (15), there were six women in class B, five in class C, seven in class D, and two in class F. The mean age of the mothers at delivery was 29.0 ± 4.1 (SD) years and the mean duration of diabetes had been 14.5 ± 7.8 years. Mean individual HbA_{1c} levels throughout pregnancy ranged from 4.9 to 9.0% (mean ± SD: 6.7 ± 1.1%), with a reference range of 4–6% in nondiabetic subjects (16). The mean gestational age at birth was 37.5 ± 1.0 weeks (range 35.6–39.0) and the mean birth weight 3,910 ± 930 g (range 2,365–6,130). Slightly more than half of the infants were boys (55%, 11 of 20). The mothers were encouraged to breast-feed their infants for at least 6 months. The study design was double blinded and the infants were randomized into two groups. Those in the intervention group were given a casein hydrolysate formula (Nutramigen, Mead Johnson, Evansville, IN) for the first 9 months of life whenever breast milk was not available. This formula is devoid of intact cow's milk proteins and ~90% of the protein load comprises peptides with a molecular weight <1,000 Da. The infants in the control group were given a regular formula (Enfamil, Mead Johnson) as supplementary milk up to the age of 9 months.

The infants were examined clinically within a few days of birth and subsequently at intervals of 3 months over the first year and then at the age of 2 years. Cord blood samples were taken at delivery for the analysis of genetic susceptibility and IDDM-associated antibodies. Follow-up samples were obtained every 3rd month during the 1st year and then at the end of the 2nd year. A blood sample taken from the mother at the end of the 1st trimester of pregnancy for rubella and syphilis screening was available for the quantification of maternal IDDM-associated antibodies. A few samples were unavailable through an oversight or because of technical problems. Altogether, there were 19 maternal samples, 18 cord blood samples, 18 samples at 3 months, 19 samples at 6 months, 17 samples at 9 months, and 18 samples at 12 and 24 months.

Genetic susceptibility. The HLA-DQB1 genotype was analyzed from cord blood specimens by a method based on the polymerase chain reaction to amplify the gene segment and by hybridization with lanthanide-labeled sequence-specific oligonucleotide probes (17). Alleles conferring disease susceptibility (HLA-DQB1*0302 and *0201) and alleles associated with protection (HLA-DQB1*0602, *0603, and *0301) were defined.

Islet cell antibody (ICA) assay. Islet cell antibodies (ICAs) were determined by a standard indirect immunofluorescence assay on sections of frozen human group O pancreas (18). Rabbit anti-human IgG (Behringwerke, Marburg, Germany) was used to detect ICA. Endpoint dilution titers were examined for the ICA-positive samples, and the results were expressed in Juvenile Diabetes Foundation units (JDF U) relative to an international reference standard. The detection limit was 2.5 JDF U. Our laboratory has participated in the International Workshops on Standardization of the ICA assay, in which its sensitivity was 100%, specificity 98%, validity 98%, and consistency 98% in the fourth round.

GAD65A assay. GAD65A was analyzed by the radioligand method described by Petersen et al. (19). Human recombinant islet GAD65 cDNA was transcribed in vitro with the vector pB1882, and the transcribed RNA translated in a methionine-free rabbit reticulocyte lysate (Promega, Madison, WI) in the presence of [^{35}S]methionine (Amersham International, Amersham Bucks, U.K.). Aliquots containing ~30,000 cpm of labeled GAD65 were incubated overnight at 4°C with serum (final dilution 1:25) in a total volume of 50 µl. Competition analysis was carried out by adding an excess of cold purified recombinant GAD65. The immunocomplexes were isolated by adding 7.5 mg of protein A-Sepharose (Pharmacia, Uppsala, Sweden) to each tube. After incubation for 2 h at 4°C, the reaction volume was transferred to a 96-well filtration system. The units were placed on a vacuum device, allowing rapid washing. After 10 washings, the bottom of each well in the filtration units was punched into a tube. Subsequently, 2.5 ml of scintillation fluid was added to each tube for counting in a scintillation counter. The results were expressed in relative units (RU), representing the specific binding as a percentage of that obtained with a positive standard serum. The cut-off limit for GAD65 antibody positivity was 6.6 RU (99th percentile in 372 subjects aged 0–19 years). The disease sensitivity of the present assay was found to be 80%, and the specificity was found to be 94%, based on the 101 samples included in the Second International GAD Antibody Workshop (20).

Insulin antibody assay. IA and insulin autoantibodies (IAAs) were measured by a modification of the liquid phase radioimmunoassay originally described by Palmer et al. (21). The results were expressed in nanounits per milliliter of insulin precipitated, where 1 nU/ml corresponds to a specific binding of 0.01%. The cut-off limit for antibody positivity in children under the age of 5 years was 68 nU/ml (99th percentile in 102 nondiabetic subjects under that age) and that for older children and adults was 57 nU/ml (99th percentile in 100 nondiabetic subjects over 5 years of age). We have participated in the International Workshops on Standardization of IAA assays and in the proficiency testing program for several years and have achieved a sensitivity of 78%, specificity of 100%, validity of 92%, and consistency of 100% in the proficiency testing program. Insulin antibodies detected in cord blood samples were considered to be IAs in response to exogenous insulin therapy, and this was confirmed by the presence of IAs in the maternal blood sample obtained at the end of the 1st trimester. IAAs were defined when antibodies were detected after at least one postnatal antibody-negative sample or after the age of 9 months.

Statistical analysis. The data were evaluated statistically by means of cross-tabulation and χ^2 statistics or Fisher's exact probability test, the unpaired Student's t test in the case of normally distributed variables, and the Mann-Whitney U test in the case of an unequal distribution. Correlation analyses were performed with Spearman's rank correlation test (r_s).

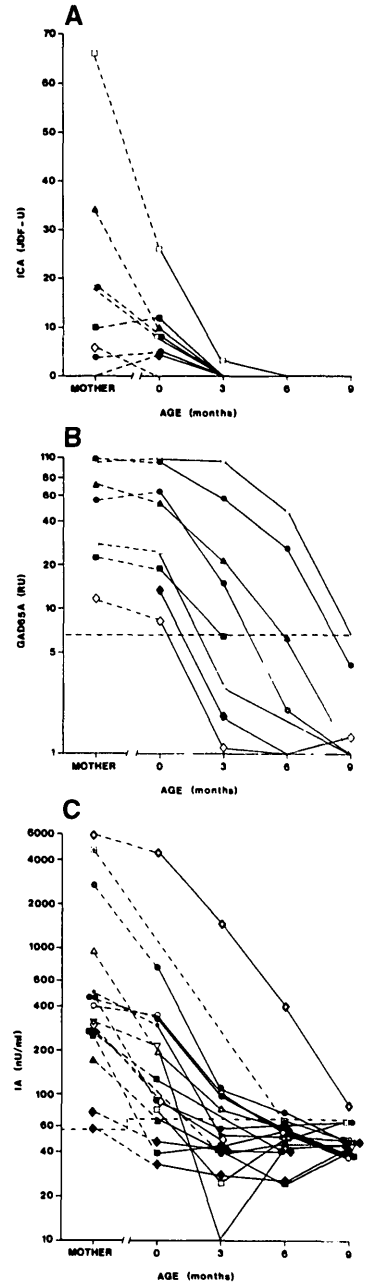

FIG. 1. Levels of ICA (*A*), GAD65A (*B*), and IA (*C*) in IDDM mothers at the end of the 1st trimester and in their infants from birth to the age of 9 months. The case presented in Fig. 3 is excluded. The vertical dashed lines in *B* and *C* mark the cut-off limit for antibody positivity.

RESULTS

Elimination of transplacentally transferred antibodies. Of the mothers with samples available, 7 out of the 19 (37%) tested positive for ICA at the end of the 1st trimester, with levels ranging from 4 to 66 JDF U (median 18). Seven infants out of 18 (39%) had ICA in their cord blood sample, with a median level of 8 JDF U (range 4–26). There was a close correlation between maternal ICA levels in early pregnancy and ICA levels in the cord blood (r_s = 0.87; P < 0.001). ICA disappeared from the peripheral circulation of all but one of the initially positive infants over the first 3 months. The one exception seroconverted to ICA negativity before the age of 6 months (Fig. 1*A*).

Eight mothers (42%) had GAD65A in early pregnancy, with levels ranging from 11.7 to 109.2 RU (median 41.7), and a similar proportion of the newborn infants (8 of 18; 44%) had transplacentally transferred GAD65A (median 38.8, range 8.2–107.3). The GAD65A levels in the maternal samples correlated closely with those in the cord blood samples (r_s = 0.87; P < 0.001). Four infants (22%) still tested positive for GAD65A at the age of 3 months. Only two infants (11%) with the highest levels in cord blood (Fig. 1*B*) remained positive at 6 months. Both of these infants became negative for GAD65A by the age of 9 months.

Of the pregnant women with IDDM, 17 out of 19 (90%) were found to be positive for IA (>57 nU/ml) in early pregnancy, with a median of 400 nU/ml (range 58–5,863). Eleven out of seventeen newborn infants (65%) tested positive for IA (>68 nU/ml) at birth, with a median of 217 nU/ml (range 79–4,531). There was a highly significant correlation between the maternal IA levels in early pregnancy and the levels of IA in cord blood (r_s = 0.86; P < 0.001). Transplacentally transferred antibodies disappeared by 9 months in all but one of the infants—the infant who had the highest initial level (Fig. 1*C*).

All but one infant of mothers without detectable antibodies at the end of the 1st trimester tested negative for antibodies in the cord blood. The only exception had an ICA level of 4 JDF U in cord blood. The mothers of infants with detectable ICA or GAD65A in cord blood had presented with IDDM at an older age (ICA: 21.6 ± 5.0 vs. 9.9 ± 6.0 years; P = 0.001; GAD65A: 19.5 ± 7.5 vs. 10.4 ± 6.2 years; P = 0.01), and they had a shorter disease duration (ICA: 7.6 ± 4.4 vs. 18.2 ± 6.8 years; P = 0.002; GAD65A 9.3 ± 6.5 vs. 17.9 ± 6.9 years; P = 0.02) than mothers of infants testing negative for ICA or GAD65A. Maternal age at delivery and metabolic control of maternal IDDM were similar in the antibody positive and negative groups. Age at diagnosis, duration of IDDM, age at delivery, and metabolic control during pregnancy were comparable between mothers of infants with and without IAs. Gestational age and birth weight were also of the same magnitude in the infants testing positive for IAs or GAD65As and those testing negative, whereas those infants testing positive for ICA had a higher gestational age (38.2 ± 0.7 vs. 37.1 ± 1.0 weeks; P = 0.02) than the ICA-negative infants.

Emergence of autoantibodies indicating β-cell damage. No child seroconverted to positivity for ICA or GAD65A before the age of 2 years, except for a boy who progressed to clinical disease at the age of 14 months. His case will be described in detail below. He also became positive for IAAs. Three additional infants tested positive for IAAs on at least one occasion over the first 12 months of their life (Fig. 2). At the age of 2 years, 5 out of 16 unaffected children (31%) were positive for IAA, with a median of 72 nU/ml (range 71–106).

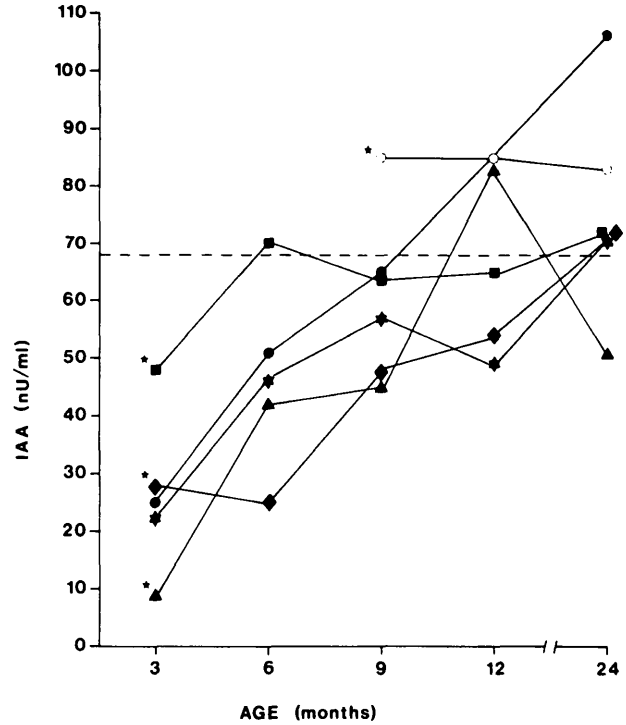

FIG. 2. Levels of IAA over the first 2 years of life in offspring of IDDM mothers. Filled symbols represent infants with increased genetic risk (HLA DQB1*0302/0201, *0302/x; x = *0302 or neutral allele, or *0201/y; y = *0201 or neutral allele), open symbols represent an infant without susceptible HLA DQB1 alleles, ★ represents infants fed with the casein hydrolysate formula as supplementary milk. The case presented in Fig. 3 is excluded. The dashed line marks the cut-off limit for IAA positivity.

Of the newborn infants, 11 of 20 (55%) had increased genetic susceptibility to IDDM. Three (15%) were heterozygous for HLA-DQB1*0302/0201, five (25%) had the *0302/x genotype (x = *0302 or neutral allele), and three (15%) had the *0201/y combination (y = *0201 or neutral allele). Four out of five subjects testing positive for IAAs at 24 months had a HLA-DQB1 genotype predisposing them to IDDM. There were no significant differences between the two formula groups in the levels of various autoantibodies at any time point analyzed after excluding the subject who progressed to IDDM nor was there any significant association between the duration of breast-feeding and the autoantibody levels at various time points.

Case report on an infant who progressed to clinical IDDM. One infant, a boy with the DQB1 genotype *0302/x, became positive for IAAs between the ages of 3 and 6 months (Fig. 3), and the level subsequently increased. Between the ages of 6 and 9 months, he seroconverted to ICA and GAD65A positivity. The ICA and IAA levels increased further after the age of 9 months, and he presented with classical symptoms of IDDM at the age of 14 months. He had been breast-fed up to the age of

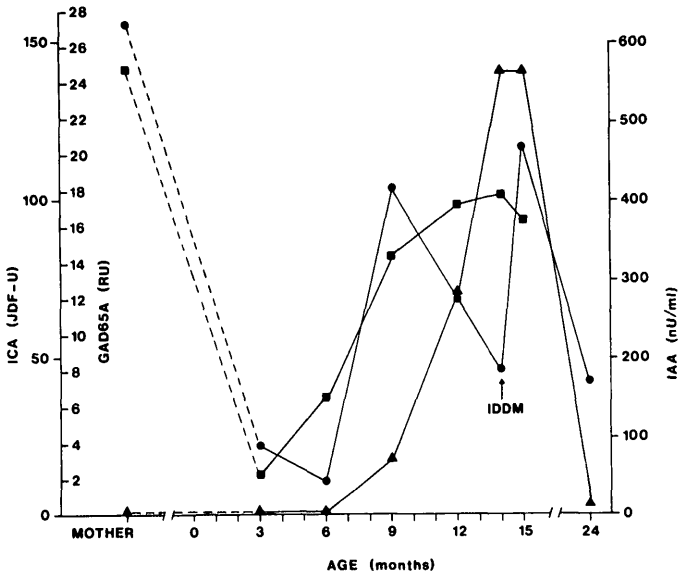

FIG. 3. Levels of ICA (▲), GAD65A (●), and IAA (■) in the infant who manifested IDDM at the age of 14 months.

35 days, but exclusively breast-fed for only 2 days. He had been given the casein hydrolysate formula up to the age of 9 months.

DISCUSSION

Our results confirm previous findings that there is a close correlation between the levels of ICA and IA in cord blood and those in the maternal circulation (22), although the maternal samples had been obtained at the end of the 1st trimester. On the other hand, it has been shown previously that IA levels in women with IDDM remain stable during pregnancy (4). We demonstrated for the first time a close correlation between the levels of GAD65A in cord blood and in the maternal circulation. These observations provide further evidence for transplacental passage of IDDM-associated antibodies from diabetic mothers to their offspring.

This prospective study with frequent follow-up visits from birth demonstrates that transplacentally transferred maternal ICAs, IAs, and GAD65A are usually eliminated from the peripheral circulation of infants of diabetic mothers over the first 6–9 months of life. Transplacentally transferred ICAs were eliminated principally over the first few months of life, only one child remaining positive after the age of 3 months. Transplacentally transferred IAs and GAD65A were eliminated before the age of 9 months, with the exception of the infant with the highest initial IA level. In general, the higher the initial level was, the longer the time required for elimination.

The appearance of IAAs before the age of 6 months and of ICAs and GAD65A before 9 months in the boy who progressed to IDDM shows that signs of autoimmune β-cell damage may emerge at a very young age. IAAs may represent a more common phenomenon of islet cell–specific autoimmunity than ICA and GAD65A in infants of mothers with IDDM, but only constantly positive increasing IAA levels appear to be markers of progressive β-cell damage. ICA and GAD65A seem to be more specific markers of significant β-cell destruction than IAA.

Given a cumulative incidence of IDDM of 2% before the age of 20 years in the offspring of mothers with IDDM (3), one can expect on average to have less than one progressor in a group of 20 subjects. The fact that the boy who presented with IDDM was given the casein hydrolysate formula is intriguing. Data on antibodies to cow's milk proteins indicated good compliance with the dietary intervention in this infant, since he had no IgG class antibodies to β-lactoglobulin or bovine serum albumin at the age of 6 and 9 months, whereas all infants on the regular formula had detectable antibodies at these time points (23). On the other hand, he was the only subject in the total series with signs of a neonatal enterovirus infection and suffered another enterovirus infection at the end of the first year (H. Hyöty, M. Lönnrot, M.K., H.K.Å., unpublished observations). We have recently found evidence that enterovirus infections may both initiate and potentiate β-cell damage (24,25). Hence, one can speculate that the neonatal enterovirus infection resulted in this case in the initiation of autoimmune β-cell destruction reflected in the emergence of IAAs before the age of 6 months and the subsequent appearance of other autoantibodies.

The main target of this pilot study was to assess the feasibility of the planned intervention program (26) to test the hypothesis that avoidance of cow's milk proteins in early infancy will reduce the risk of subsequent IDDM. Most of the data on IDDM-associated antibodies in the present report are by necessity descriptive. Because of the small population, the only scientifically sound conclusion concerning the basic hypothesis is that the dietary intervention did not provide complete protection against IDDM. On the other hand, the estimates of the total population needed for the intervention trial proper have been based on the assumption that a dietary intervention of this kind would result in a 33% reduction in the incidence of IDDM.

ACKNOWLEDGMENTS

This research was supported by the Sigrid Jusélius Foundation, the Juvenile Diabetes Foundation International (grant 192612), the Liv and Hälsa Foundation, the Novo Nordisk Insulin Foundation, and the Dorothea Olivia, Karl Walter, and Jarl Walter Perklén Foundation.

We thank Marja Salonen and Tarja Tenkula for excellent collaboration and Päivi Ronkainen, Riitta Päkkilä, and Susanna Heikkilä for their skillful technical assistance. We are grateful to the Mead Johnson Nutritional Group and Dr. James W. Hansen for providing us with the study formulas.

REFERENCES

1. Warram JH, Krolewski AS, Gottlieb MS, Kahn CR: Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and fathers. *N Engl J Med* 311:149–152, 1984
2. Warram JH, Krolewski AS, Kahn CR: Determinants of IDDM and perinatal mortality in children of diabetic mothers. *Diabetes* 37:1328–1334, 1988
3. Tuomilehto J, Lounamaa R, Tuomilehto-Wolf E, Reunanen A, Virtala E, Kaprio EA, Åkerblom HK, the Childhood Diabetes in Finland (DiMe) Study Group: Epidemiology of childhood diabetes mellitus in Finland—background of a nationwide study of type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 35:70–76, 1992
4. Di Mario U, Fallucca F, Gargiulo P, Tiberti C, Scardetellato A, Arduini P,

Pachi A, Andreani D: Insulin-anti-insulin complexes in diabetic women and their neonates. *Diabetologia* 27:83–86, 1984
5. Tingle AJ, Lim G, Wright VJ, Dimmick JE, Hunt JA: Transplacental passage of islet cell antibody in infants of diabetic mothers. *Pediatr Res* 13:1323–1325, 1979
6. Lernmark Å: Molecular biology of IDDM. *Diabetologia* 37:S73–S81, 1994
7. Thorsby E, Ronningen KS: Particular HLA-DQ molecules play a dominant role in determining susceptibility or resistance to type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 36:371–377, 1993
8. Reijonen H, Ilonen J, Knip M, Åkerblom HK: HLA-DQB1 alleles and absence of Asp 57 as susceptibility factors of IDDM in Finland. *Diabetes* 40:1640–1644, 1991
9. Reijonen H, Vähäsalo P, Karjalainen J, Ilonen J, Åkerblom HK, Knip M, the Childhood Diabetes in Finland (DiMe) Study Group: HLA-DQB1 genotypes and islet cell antibodies in the identification of siblings at risk for insulin-dependent diabetes (IDDM) in Finland. *J Autoimmun* 7:675–686, 1994
10. Åkerblom HK, Knip M, Hyöty H, Virtanen SM, Savilahti E, Ilonen J: Environmental risk factors of insulin-dependent diabetes mellitus. *Arct Med Res* 53 (Suppl. 2):163–167, 1994
11. Gorsuch AN, Lister J, Dean BM, Spencer KM, McNally JM, Bottazzo GF, Cudworth AG: Evidence for a long prediabetic period in type 1 (insulin-dependent) diabetes mellitus. *Lancet* ii:1363–1365, 1981
12. Knip M, Vähäsalo P, Karjalainen J, Lounamaa R, Åkerblom HK, the Childhood Diabetes in Finland Study Group: Natural history of preclinical IDDM in high risk siblings. *Diabetologia* 37:388–393, 1994
13. Virtanen SM, Räsänen L, Ylönen K, Aro A, Clayton D, Langholz B, Pitkäniemi J, Savilahti E, Lounamaa R, Tuomilehto J, Åkerblom HK, the Childhood Diabetes in Finland Study Group: Early introduction of dairy products associated with increased risk of IDDM in Finnish children. *Diabetes* 42:1786–1790, 1993
14. Gerstein HC: Cow's milk exposure and type 1 diabetes mellitus: a critical overview of the literature. *Diabetes Care* 17:13–19, 1994
15. White P: Pregnancy and diabetes. *Med Clin N Am* 49:1015–1024, 1965
16. Stenman U-H, Pesonen K, Ylinen K, Huhtala M-L, Teramo K: Rapid chromatographic quantitation of glycosylated haemoglobins. *J Chromatogr* 297:327–332, 1984
17. Sjöroos M, Iitiä A, Ilonen J, Reijonen H, Lövgren T: Triple-label hybridization assay for type-1 diabetes-related HLA alleles. *BioTechniques* 18:870–877, 1995
18. Bottazzo GF, Florin-Christensen A, Doniach D: Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* ii:1279–1982, 1974
19. Petersen JS, Hejnaes KR, Moody A, Karlsen AE, Marchall MO, Hoier-Madsen M, Boel E, Michelsen BK, Dyrberg T: Detection of GAD65 antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. *Diabetes* 43:459–467, 1994
20. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Paquette TL: Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science* 222:1337–1339, 1983
21. Schmidli RS, Colman PG, Bonifacio E, Participating Laboratories: Disease sensitivity and specificity of 52 assays for glutamic acid decarboxylase antibodies: the Second International GADAb Workshop. *Diabetes* 44:636–640, 1995
22. Ziegler A-G, Hillebrand B, Rabl W, Mayrhofer M, Hummel M, Mollenhauer 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
23. Vaarala O, Saukkonen T, Savilahti E, Klemola T, Åkerblom HK: Development of immune response to cow's milk proteins in infants receiving cow's milk or hydrolysed formula. *J Allergy Clin Immunol* 96:917–923, 1995
24. Hyöty H, Hiltunen M, Knip M, Laakkonen M, Vähäsalo P, Karjalainen J, Koskela P, Roivainen M, Leinikki P, Hovi T, Åkerblom HK, the Childhood Diabetes in Finland (DiMe) Study Group: A prospective study of the role of Coxsackie B and other enterovirus infections in the pathogenesis of IDDM. *Diabetes* 44:652–657, 1995
25. Hyöty H, Hiltunen M, Knip M, Ilonen J, Laakkonen M, Roivainen M, Vähäsalo P, Reijonen H, Karjalainen J, Koskela P, Leinikki P, Hovi T, Åkerblom HK, the DiMe Study Group: Induction of beta-cell damage by enterovirus infections: a prospective study. *Diabetologia* 38 (Suppl. 1):A16, 1995
26. Åkerblom HK, Savilahti E, Saukkonen TT, Paganus A, Virtanen SM, Teramo K, Knip M, Ilonen J, Reijonen H, Karjalainen J, Vaarala O, Reunanen A: The case for elimination of cow's milk in early infancy in the prevention of type 1 diabetes: the Finnish experience. *Diabetes Metab Rev* 9:269–278, 1993