Rapid Publication

Transmission of Maternal Islet Antibodies and Risk of Autoimmune Diabetes in Offspring of Mothers With Type 1 Diabetes

Kerstin Koczwara,[1] Ezio Bonifacio,[2] and Anette-Gabriele Ziegler[1]

It is suggested that the maternal transmission of islet autoantibodies increases the risk of autoimmune diabetes in mice. The aim of this study was to determine whether fetal exposure to islet autoantibodies modified the risk of type 1 diabetes in humans. Islet autoantibodies were measured at birth in 720 offspring of mothers with type 1 diabetes. Offspring were prospectively followed for the development of multiple islet autoantibodies and diabetes. Offspring who were GAD or IA-2 autoantibody positive at birth (n = 678) had significantly lower risks for developing multiple islet autoantibodies (5-year risk 1.3%) and diabetes (8-year risk 1.1%) than offspring who were islet autoantibody negative at birth (5.3%, P = 0.008; and 3%, P = 0.04, respectively). Risk remained reduced after adjustment for birth weight, gestational age, or maternal diabetes duration (adjusted hazards ratio 0.25, P = 0.007 for multiple islet autoantibodies; 0.25, P = 0.04 for diabetes). Protection in offspring with islet autoantibodies at birth was most striking in offspring without the HLA *DRB103/*DRB1**04-*DQB*1*0302 genotype. Maternal transmission of antibodies to exogenous insulin did not affect diabetes risk in offspring. These findings suggest that fetal exposure to islet autoantibodies in children born to mothers with type 1 diabetes may be protective against future islet autoimmunity and diabetes. *Diabetes* 53:1–4, 2004**

Islet autoantibodies precede the clinical development of autoimmune diabetes in humans and in the nonobese diabetic (NOD) mouse. Islet autoantibodies can be transferred through the placenta from islet antibody–positive mothers to their offspring (1–4). The influence of these maternally transmitted islet antibodies on the ontogeny of islet autoimmunity and type 1 diabetes in the offspring remains unknown. In the NOD mouse, it has recently been shown that elimination of maternally transmitted immunoglobulin prevented spontaneous diabetes in progeny, suggesting that humoral factors present during gestation, including immunoglobulin, and autoantibodies potentially play an important role in the pathogenesis of β-cell destruction (5,6). The BABYDIAB study presents an opportunity to test this hypothesis in humans because children of mothers with type 1 diabetes are followed from birth for the development of islet autoantibodies and diabetes. We examined whether the transmission of islet autoantibodies (GAD antibodies [GADAs] and IA-2 antibodies [IA-2As]) and/or antibodies to exogenous antigen (insulin antibodies) from the type 1 diabetic mother to children during pregnancy modified their risk of developing diabetes-associated islet autoimmunity. The findings suggest that fetal exposure to islet autoantibodies does not increase the risk of diabetes development and may protect against future islet autoimmunity.

RESEARCH DESIGN AND METHODS

Offspring of parents with type 1 diabetes (the BABYDIAB study). BABYDIAB prospectively follows offspring of mothers and/or fathers with type 1 diabetes from birth. Cord blood is obtained at birth, and venous blood samples are obtained at age 9 months, and at ages 2, 5, 8, and 11 years (7). Recruitment began in July 1989 and ended in November 2000, and follow-up has continued thereafter. A total of 1,610 children of a parent with type 1 diabetes were recruited at birth and participated in the 9-month follow-up. These included 1,030 children of mothers with type 1 diabetes. Cord blood for determination of all three antibodies (insulin antibody, GADA, and IA-2A) at birth was available in 720 of 1,030 offspring of mothers with type 1 diabetes and in 285 of 580 offspring of fathers with type 1 diabetes and nondiabetic mothers. The cord blood volume was insufficient for islet antibody determination in the remaining 290 offspring of mothers with type 1 diabetes and 295 offspring of fathers with type 1 diabetes. After birth, insulin autoantibodies (IAAs), GADAs, and IA-2As were measured in samples from all scheduled follow-up visits. The median follow-up time from birth to last sample was 6.5 years (range 0.75–12.5 years), for a total of 9,773 person-years. HLA DR and DQ genotypes were determined in 602 of the 720 offspring with birth islet autoantibody measurements. Type 1 diabetes diagnosis was defined according to World Health Organization (WHO) criteria. All parents gave written informed consent to participate in BABYDIAB. The study was approved by the ethical committee of Bavaria, Germany (Bayerische Landesärztekammer no. 95357).

Islet antibody measurement. Insulin antibody(A), GADA, and IA-2A were determined by radiobinding assays as previously described (7,8). The 99th percentile of control children were used to define the upper limits of normal and corresponded to 8.5 local units/ml or 25 WHO units/ml for GADA, 2.5 local units/ml or 4 WHO units/ml for IA-2A, and 1.5 local units/ml for IAA. The assays had sensitivities and specificities of 80 and 94% (GADA), 58 and 100% (IA-2A), and 30 and 98% (IAA), respectively, in the First Diabetes Autoanti-

From the [1]Diabetes Research Institute and 3rd Medical Department, Krankenhaus München-Schwabing, Munich, Germany; and the [2]Istituto Scientifico San Raffaele, Milan, Italy.

Address correspondence and reprint requests to Prof. Dr. Anette-Gabriele Ziegler, Diabetes Research Institute, Koelner Platz 1, D-80804 Munich, Germany. E-mail: anziegler@lrz.uni-muenchen.de.

Received for publication 27 August 2003 and accepted in revised form 20 October 2003.

Posted on the World Wide Web at http://diabetes.diabetesjournals.org on 3 November 2003.

GADA, GAD antibody; IA-2A, IA-2 antibody; IAA, insulin autoantibody; WHO, World Health Organization.

TABLE 1
Cumulative antibody and diabetes risk in offspring of mothers with type 1 diabetes relative to antibody status at birth

Antibody status at birth	Multiple islet antibodies (% at 5 years)*	P†	Adjusted HR‡	P§	Diabetes (% at 8 years)*	P†	Adjusted HR‡	P§
Insulin antibodies (*n* [%])								
Positive (620 [86])	3.1 ± 0.7	0.7	1.4 (0.3–6.2)	0.63	2.3 ± 0.7	0.15	NA‖	0.93
Negative (100 [14])	2.1 ± 1.5		reference		0			
GAD autoantibodies (*n* [%])								
Positive (401 [56])	1.6 ± 0.6	0.1	0.4 (0.1–1.1)	0.08	1.3 ± 0.6	0.21	0.4 (0.1–1.3)	0.11
Negative (319 [44])	4.0 ± 1.2		reference		2.3 ± 1.0		reference	
IA-2 autoantibodies (*n* [%])								
Positive (267 [37])	1.3 ± 0.7	0.08	0.3 (0.1–1.2)	0.10	1.5 ± 0.9	0.44	0.6 (0.2–2.2)	0.46
Negative (453 [63])	3.5 ± 0.9		reference		1.9 ± 0.7		reference	
GAD or IA-2 autoantibodies (*n* [%])								
Positive (478 [66])	1.3 ± 0.5	0.008	0.25 (0.1–0.7)	0.007	1.1 ± 0.5	0.04	0.25 (0.1–0.8)	0.02
Negative (242 [34])	5.3 ± 1.6		reference		3.0 ± 1.3		reference	

Data are means ± SE or HR (95% CI). *Life table risk; †*P* values calculated using the log-rank test; ‡HR adjusted for maternal diabetes duration, birth weight, and gestational age; §*P* values calculated for adjusted HR against reference cell; ‖unable to calculate HR.

bodies Standardization Program Proficiency Workshop (9). All measurements were performed on coded samples that were operator blinded.

HLA typing. HLA-DRB1, HLA-DQA1, and HLA-DQB1 alleles were typed using PCR-amplified DNA and nonradioactive sequence-specific oligonucleotide probes as previously described (10).

Statistical analysis. Time-to-event methods (life table analysis and Cox proportional hazards model) were used to analyze islet autoantibody and diabetes development in the offspring. Positive autoantibody outcome in children was defined as islet autoantibodies to at least two autoantigens (insulin, GAD, or IA-2) in at least two consecutive samples (persistent multiple islet autoantibody positive). Islet autoantibodies against a single autoantigen have not been demonstrated to be associated with diabetes development, and children with single islet autoantibodies were therefore considered negative. Children with autoantibodies in one sample and no subsequent sample for confirmation were also considered negative. The age of onset of islet autoantibody positivity was defined as the age at the first positive sample. Analyses considered censoring in losses to follow-up and in autoantibody-negative children at their age during the follow-up visit of their last antibody-negative sample. Offspring of mothers with type 1 diabetes were categorized according to their antibody status at birth as insulin antibody positive and negative, GADA positive and negative, IA-2A positive and negative, and autoantibody (GADA or IA-2A) positive or negative. Hazards ratios (HRs) were calculated using Cox's proportional hazards model and were adjusted for the duration of type 1 diabetes in the mother (<10 years or >10 years), low birth weight (<2,700 g), and short gestation age (<36 weeks). All *P* values were two-tailed. All statistical analyses were performed using SPSS (Chicago, IL).

RESULTS

Prevalence of islet antibodies in cord blood of offspring from mothers with type 1 diabetes. At birth, insulin antibodies were detected in 620 of 720 (86%) offspring of type 1 diabetic mothers, GADAs were in 401 of 720 (55.7%) offspring, and IA-2As in 267 of 720 (37%) offspring (Table 1). GADAs or IA-2As were detected at birth in 478 (66%) offspring. Of the 720 offspring, 45 (6.3%) had no islet antibodies in their cord blood, 54 (7.5%) had GADAs (44%) and/or IA-2As (25%) without insulin antibodies, 196 (27.2%) had insulin antibodies only, and 424 (58.9%) had GADAs and/or IA-2As together with insulin antibodies. One of the 285 offspring of fathers with type 1 diabetes had IAAs at birth, none had IA-2As, and none had GADAs.

Relationship of birth islet antibody status and subsequent development of diabetes-associated autoimmunity in offspring. Thirty-one offspring of type 1 diabetic mothers developed multiple islet autoantibodies during early childhood, and 16 of these developed diabetes. Autoantibody status at birth significantly affected diabetes risk in offspring of mothers with type 1 diabetes. Offspring who were islet autoantibody (GADA or IA-2A) positive at birth had a significantly lower risk for the subsequent development of multiple islet autoantibodies (1.3% by age 5 years) and diabetes (1.1% by age 8 years) than offspring who were islet autoantibody negative at birth (5.3%, $P = 0.008$ for multiple islet autoantibodies; 3.0%, $P = 0.04$ for diabetes) (Table 1) (Fig. 1). Risks were

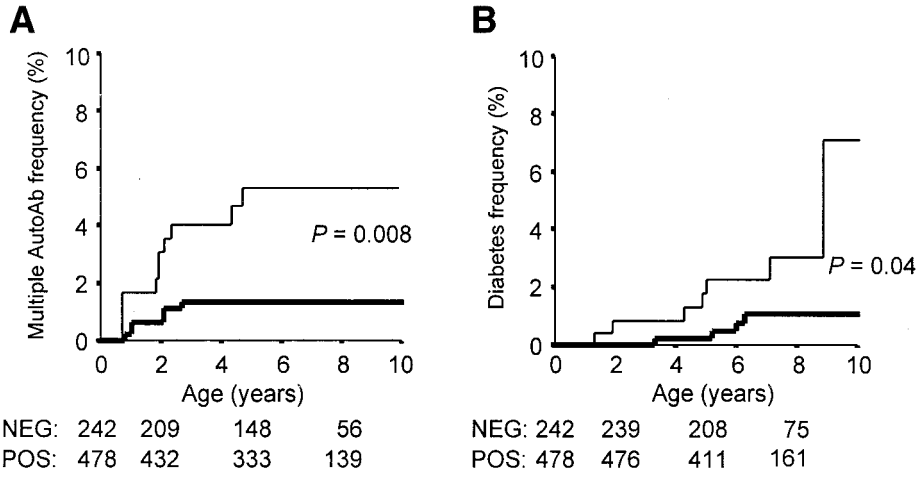

FIG. 1. Life table cumulative risks for developing persistent multiple islet autoantibodies (*A*) and for developing diabetes (*B*) in offspring of mothers with type 1 diabetes. Risks in offspring who were GAD or IA-2 autoantibody positive at birth are represented by the thick solid line, and risks in offspring who were GAD and IA-2 autoantibody negative at birth are represented by the thin line. Numbers under the abscissa indicate the number of offspring who were GAD and IA-2 autoantibody negative at birth (NEG) and who were GAD or IA-2 autoantibody positive at birth (POS) remaining at birth and at ages 2, 5, and 8 years.

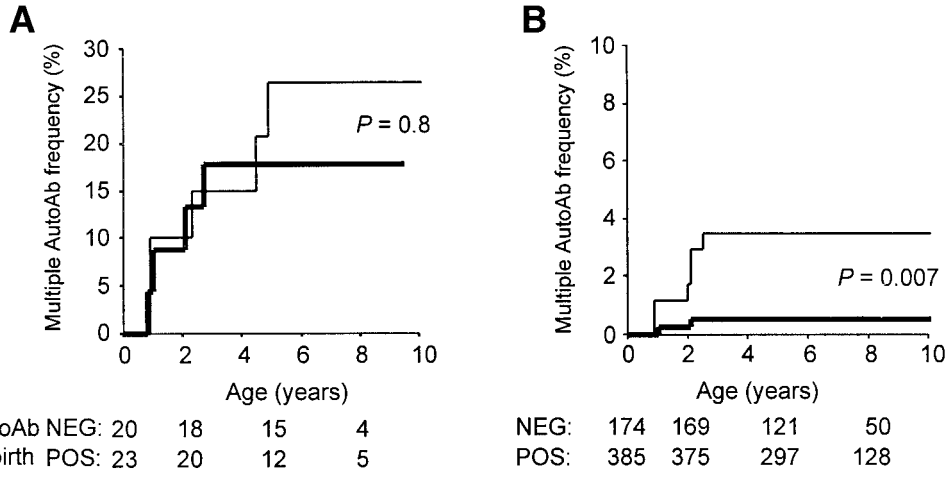

FIG. 2. Life table cumulative risks for developing persistent multiple islet autoantibodies in offspring with the HLA DR3/DR4-DQ8 genotype (*A*) and in offspring without the HLA DR3/DR4-DQ8 genotype (*B*). Risks in offspring who were GAD or IA-2 autoantibody positive at birth are represented by the thick solid line, and risks in offspring who were GAD and IA-2 autoantibody negative at birth are represented by the thin line. Numbers under the abscissa indicate the number of offspring in offspring who were GAD and IA-2 autoantibody negative at birth (NEG) and who were GAD or IA-2 autoantibody positive at birth (POS) remaining at birth and at ages 2, 5, and 8 years.

also reduced in the offspring with GADAs or IA-2As at birth after adjustment for maternal diabetes duration, gestational age, and birth weight (adjusted HR for developing multiple islet autoantibodies 0.25, $P = 0.007$; adjusted HR for developing diabetes 0.25, $P = 0.02$). The effect of birth autoantibody status on diabetes risk was most striking in children who did not have the high-risk HLA *DRB1**03/*DRB1**04-*DQB*1*0302 genotype (0.5 vs. 3.5% multiple islet autoantibodies by age 5 years, $P = 0.007$; 0.3 vs. 2.4% diabetes by age 8 years, $P = 0.05$) and was negligible in HLA *DRB1**03/*DRB1**04-*DQB*1*0302–positive children (17.8 vs. 26.3% multiple islet autoantibodies, $P = 0.8$; 18.4 vs. 15.6% diabetes, $P = 0.7$) (Fig. 2). No significant difference in the risk for developing multiple islet autoantibodies or diabetes was found between offspring with or without cord blood insulin antibodies (Table 1). The risk of developing multiple islet autoantibodies was 4 ± 0.9% (mean ± SE) in the 284 offspring of fathers with type 1 diabetes who were islet autoantibody negative at birth.

DISCUSSION

Antibodies bind antigens and can act as cell-surface receptors on B-cells or monocytes and dendritic cells to facilitate or enhance presentation of antigen to T-cells (11–14). As a result, antibodies may play a role in the pathogenesis of autoimmune disease (15). In autoimmune diabetes, however, it has generally been considered that autoantibodies are purely markers of disease because blocking B-cell function in experimental models does not affect the development of disease (16), and removal of immunoglobulin from type 1 diabetic patients has only a minimal effect on disease severity (17,18). While these observations show that autoantibodies alone are insufficient for diabetes development, more recent studies (15,19–21) have demonstrated that B-cell–deficient NOD mice have little insulitis and have a markedly reduced diabetes incidence, indicating that B-cells and perhaps antibodies play a role in the initiation of diabetes. This hypothesis was recently reinforced by the finding (5) that the removal of immunoglobulin during gestation markedly reduced the incidence of diabetes. It remains to be determined whether these observations were due specifically to the presence or absence of autoantibodies to islet antigens in both the NOD mouse and humans.

In humans, the BABYDIAB study provided an opportunity to examine whether fetal and neonatal exposure to autoantibodies and to endogenous autoantigen (GAD or IA-2) or to antibodies against exogenously administered autoantigen (insulin) modified diabetes risk. The BABYDIAB study is the largest study of offspring of parents with type 1 diabetes and includes >1,000 offspring of mothers with type 1 diabetes. We and others have previously shown that islet antibodies in cord blood of offspring from type 1 diabetic mothers correlate highly with the level of antibodies found in maternal blood at delivery, suggesting the transmission of these antibodies through the placenta (1,2) and that these maternally transmitted antibodies usually become undetectable within the first year of life (3,4). Here we determined the prevalence of islet antibodies at birth and followed offspring for the development of persistent diabetes-associated multiple islet autoantibodies and for the development of diabetes. Antibodies to exogenously administered insulin were detected at birth in 86% of offspring of type 1 diabetic mothers, and 66% of offspring had autoantibodies to GAD and/or IA-2 at birth. We found that the presence or absence of insulin antibodies did not affect the risk of developing diabetes or diabetes-associated autoantibodies. Remarkably, however, offspring with autoantibodies at birth had a significantly lower diabetes risk than offspring who were autoantibody negative at birth. The decreased risk did not appear secondary to potential confounders, such as maternal diabetes duration, birth weight, and gestational age.

Assuming that antibodies detected at birth are representative of antibody titers during pregnancy, the findings in the BABYDIAB offspring suggest that fetal exposure to GAD and/or IA-2 autoantibodies may protect against subsequent diabetes. Consistent with this observation is the overall decreased diabetes risk in offspring of type 1 diabetic mothers compared with that of offspring of type 1 diabetic fathers and nondiabetic mothers (22,23) and the previous report (24) of reduced development of islet autoimmunity in offspring of mothers with type 1 diabetes compared with children of fathers with type 1 diabetes. Although many factors are likely to contribute to this difference, it is possible that the fetal autoantibody exposure that occurs in the majority of offspring of type 1 diabetic mothers plays a role in reducing their diabetes risk. Such protection could be due to a more efficient

elimination of autoreactive T-cell clones by antibody-mediated presentation of autoantigen during fetal and neonatal life or to a state of immune ignorance against autoantigen by antibody-mediated blocking or masking the presentation of relevant autoantigen peptides (25). Interestingly, the protection conferred by fetal exposure to autoantibodies was most striking in children who did not have the typical high diabetes risk HLA *DRB1**03/*DRB1**04-*DQB*1*0302 genotype, suggesting that the mechanism is unlikely to require HLA restriction of antigen presentation.

Overall, our findings in humans do not support the hypothesis that fetal exposure to islet autoantibodies increases diabetes risk and, if these observations are confirmed, suggest that fetal autoantibody exposure may protect from future endogenous islet autoimmunity and diabetes.

ACKNOWLEDGMENTS

This study was supported by grants from the Juvenile Diabetes Research Foundation (JDRF nos. 1-2000-619 and 1-2003-646) and the Charlotte-Fiévet-Stiftung. This forms part of the dissertation of K.K. at the Technical University of Munich.

The authors thank Annette Knopff, Karolina von Dalwigk, Ulrike Mollenhauer, Heike Naserke, Michael Hummel, Markus Walter, and Doris Huber for their expert help in data collection and antibody measurements. The authors also thank all of the obstetric departments, pediatricians, and family doctors in Germany who participated in the BABYDIAB study.

REFERENCES

1. Ziegler AG, 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
2. Roll U, Christie MR, Fuchtenbusch M, Payton MA, Hawkes CJ, Ziegler AG: Perinatal autoimmunity in offspring of diabetic parents: the German multicenter BABY-DIAB study: detection of humoral immune responses to islet antigens in early childhood. *Diabetes* 45:967–973, 1996
3. Hamalainen AM, Ronkainen MS, Akerblom 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
4. Naserke HE, Bonifacio E, Ziegler AG: Prevalence, characteristics and diabetes risk associated with transient maternally acquired islet antibodies and persistent islet antibodies in offspring of parents with type 1 diabetes. *J Endocrinol Metab* 86:4826–4833, 2001
5. 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 nonobese diabetic mice. *Nat Med* 8:399–402, 2002
6. von Herrath M, Bach JF: Juvenile autoimmune diabetes: a pathogenic role for maternal antibodies? *Nat Med* 8:331–333, 2002
7. Ziegler AG, Hummel M, Schenker M, Bonifacio E: Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study. *Diabetes* 48:460–468, 1999
8. Naserke HE, Bonifacio E, Ziegler AG: Immunoglobulin G insulin antibodies in BABYDIAB offspring appear postnatally: sensitive early detection using protein A/G-based radiobinding assay. *J Endocrinol Metab* 84:1239–1243, 1999
9. Bingley PJ, Bonifacio E, Mueller PW: Diabetes Antibody Standardization Program: first assay proficiency evaluation. *Diabetes* 52:1128–1136, 2003
10. Walter M, Albert E, Conrad M, Keller E, Hummel M, Ferber K, Barratt BJ, Todd JA, Ziegler AG, Bonifacio E: IDDM2/insulin VNTR modifies risk conferred by IDDM1/HLA for development of type 1 diabetes and associated autoimmunity. *Diabetologia* 46:712–720, 2003
11. Manca F, Kunkl A, Fenoglio D, Fowler A, Sercarz E, Celada F: Constrains in T-B cooperation related to epitope topology on E. coli beta-galactosidase. I: the fine specificity of T cells dictates the fine specificity of antibodies directed to conformation-dependent determinants. *Eur J Immunol* 15:345–350, 1985
12. Lin RH, Mamula MJ, Hardin JA, Janeway CA Jr: Induction of autoreactive B cells allows priming of autoreactive T cells. *J Exp Med* 173:1433–1439, 1991
13. Watts C, Lanzavecchia A: Suppressive effect of antibody on processing of T cell epitopes. *J Exp Med* 178:1459–1463, 1993
14. Simitsek PD, Campbell DG, Lanzavecchia A, Fairweather N, Watts C: Modulation of antigen processing by bound antibodies can boost or suppress class II major histocompatibility complex presentation of different T cell determinants. *J Exp Med* 181:1957–1963, 1995
15. Noorchashm H, Noorchashm N, Kern J, Rostami SY, Barker CF, Naji A: B-cells are required for the initiation of insulitis in nonobese diabetic mice. *Diabetes* 46:941–946, 1997
16. Bendelac A, Boitard C, Bedossa P, Bazin H, Bach JF, Carnaud C: Adoptive T cell transfer of autoimmune nonobese diabetic mouse diabetes does not require recruitment of host B lymphocytes. *J Immunol* 141:2625–2628, 1988
17. Seidel DK, Geiss HC, Donner MG, Ritter MM, Schwandt P, Koll RA, Standl E, Ziegler AG: Course of islet autoantibody titers during Ig-immunoadsorption in a patient with newly diagnosed type 1 diabetes. *J Autoimmun* 11:273–277, 1998
18. Ludvigsson J, Heding L, Lieden G, Marner B, Lernmark A: Plasmapheresis in the initial treatment of insulin-dependent diabetes mellitus in children. *Br Med J (Clin Res Ed)* 286:176–178, 1983
19. Forsgren S, Andersson A, Hillron V, Soderstrom A, Holmberg D: Immunoglobulin-mediated prevention of autoimmune diabetes in the non-obese diabetic (NOD) mouse. *Scand J Immunol* 34:445–451, 1991
20. Serreze DV, Chapman HD, Varnum DS, Hanson MS, Reifsnyder PC, Richard SD, Fleming SA, Leiter EH, Shultz LD: B lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new "speed congenic" stock of NOD.Ig mu null mice. *J Exp Med* 184:2049–2053, 1996
21. Silveira PA, Johnson E, Chapman HD, Bui T, Tisch RM, Serreze DV: The preferential ability of B lymphocytes to act as diabetogenic APC in NOD mice depends on expression of self-antigen-specific immunoglobulin receptors. *Eur J Immunol* 32:3657–3666, 2002
22. Warram JH, Krolewski AS, Gottlieb MS, Kahn CR: Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. *N Engl J Med* 311:149–152, 1984
23. Pociot F, Norgaard K, Hobolth N, Andersen O, Nerup J: A nationwide population-based study of the familial aggregation of type 1 (insulin-dependent) diabetes mellitus in Denmark: Danish Study Group of Diabetes in Childhood. *Diabetologia* 36:870–875, 1993
24. Yu L, Chase HP, Falorni A, Rewers M, Lernmark A, Eisenbarth GS: Sexual dimorphism in transmission of expression of islet autoantibodies to offspring. *Diabetologia* 38:1353–1357, 1995
25. Kurts C, Sutherland RM, Davey G, Lew AM, Blanas E, Carbone F, Miller JFAP, Heath WR: CD8 T cell ignorance or tolerance to islet antigens depends on antigen dose. *Proc Natl Acad Sci U S A* 96:12703–12707, 1999