Insulin Antibody Response to a Short Course of Human Insulin Therapy in Women With Gestational Diabetes

MONTSERRAT BALSELLS, MD
ROSA CORCOY, MD, PHD
DÍDAC MAURICIO, MD, PHD
JOSEFA MORALES, MSC
APOLONIA GARCÍA-PATTERSON, MD
GEMMA CARRERAS, MD
MANUEL PUIG-DOMINGO, MD, PHD
ALBERTO DE LEIVA, MD, PHD

OBJECTIVE — To assess the insulin antibody (IA) response to human insulin (HI) therapy in women with gestational diabetes.

RESEARCH DESIGN AND METHODS — IAs were measured by a competitive radiobinding assay in 50 women with gestational diabetes before and during treatment with HI and after delivery. At delivery, 15 maternal-cord blood sample pairs were analyzed for IA. As a reference, we searched for IA in 25 new-onset type I diabetic patients, before and at 3, 6, and 12 months after insulin therapy.

RESULTS — Insulin autoantibodies (IAAs) were detected in 1 of 50 women with gestational diabetes and 4 of 16 type I diabetic patients ($P < 0.05$). At the end of pregnancy after 9.3 ± 6.8 weeks on insulin therapy, 22 of 50 (44%) women with gestational diabetes became IA^+ and 4 additional women were found to be positive 2 months postpartum. After 3 months on insulin, type I diabetic patients showed a higher rate of IA positivity (92%, $P < 0.001$). IA titers at the end of pregnancy were associated with the cumulative insulin dose ($r = 0.29$, $P < 0.05$). Postpartum, IA disappeared slowly in most IA^+ women, but two women still showed IA 2 years after delivery. Titers in cord blood were strongly related to those in maternal blood ($r = 0.74$, $P < 0.01$). The rate of adverse fetal outcome did not differ in IA^- and IA^+ mothers (27 vs. 40%, NS).

CONCLUSIONS — HI is immunogenic, and a short course of HI therapy induces IA in ~50% of women with gestational diabetes and 92% of type I diabetic patients. In women with gestational diabetes, insulin dose is slightly associated with IA titers. These IAs apparently cross the placenta. Fetal outcome does not differ according to the maternal IA status, and IAs disappear gradually after delivery but may remain positive for 2 years after delivery.

Insulin antibodies (IAs) at high titers were detected in almost all diabetic patients receiving early insulin preparations (1), and immunological reactions were observed in a significant percentage of patients (2). Availability of highly purified insulin preparations has decreased the rate of IA positivity after treatment, and the IA-linked adverse reactions have become rare (3,4). Despite the theoretical lack of immunogenicity of human insulin (HI), most studies have found that it continues to be immunogenic and patients who have only received HI also develop IA (5,6).

There is a consensus that normoglycemia is important in women with gestational diabetes (7), with a variable percentage of these women requiring insulin to achieve it (8). Since IAs have been associated with materno-fetal morbidity in insulin-treated diabetic mothers, HI has been preferred over animal insulin preparations for pregnancy use (9,10). Nevertheless, even in diabetic pregnant women with IAs, pregnancy outcome is more related to glycemic control than to IAs (11). Little is known about the immunologic response to a transient course of insulin therapy in women with gestational diabetes (6). In the present study, we have attempted to assess the IA response in HI-treated women with gestational diabetes, treatment variables associated with IA development, to compare the IA response with that of new-onset type I diabetic patients and to assess if the fetal outcome differs according to the IA status of the mother.

RESEARCH DESIGN AND METHODS

— In our center, pregnant women undergo universal gestational diabetes screening, gestational diabetes being diagnosed according to National Diabetes Data Group criteria (6). All women with gestational diabetes are instructed in using a normocaloric diet and in self-monitoring blood glucose at least four times a day (fasting, preprandial, and 1-h postprandial). Insulin therapy is initiated when fasting or preprandial capillary blood glucose is ≥90 mg/dl and/or 1-h postprandially ≥120 mg/dl more than twice per week. This study includes 50 women with gestational diabetes who required insulin to achieve glycemic goals. None had received insulin before, and only HI (Actrapid HM and Monotard HM) was used. Insulin was injected by conventional devices, and weekly insulin dose was recorded. IAs were measured before insulin therapy one or more times during treatment (along with samples drawn for clinical purposes) and postpartum.

The rate of positivity for IAs was calculated for every 3-week interval after insulin was started. When in an interval, a sample was not available, the IA^- or IA^+ status was considered to be the same of the former and later IA measurements when concordant. In the case of a previous IA^- sample and a following IA^+ sample, the patient was considered to be negative in the intermediate period. For the postpartum evaluation, patients were considered to be IA^+ until the IA^- status was demonstrated. At delivery, 15 maternal-cord blood sample pairs were drawn to determine IA.

From the Division of Endocrinology, Diabetes and Nutrition, Hospital de Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain.

Address correspondence and reprint requests to Rosa Corcoy, MD, PhD, Servei d'Endocrinologia, Hospital de Sant Pau, Avda. Sant Antoni Mª Claret 167, Barcelona 08025, Spain. E-mail: hsp.endocri@bcn.servicom.es.

Received for publication 22 November 1996 and accepted in revised form 2 April 1997.

IA, insulin antibody; IAA, insulin autoantibody; ICA, islet cell antibody; HI, human insulin.

Table 1—*IAs in women with gestational diabetes during insulin therapy and after delivery*

IAs during insulin therapy			IAs after delivery		
Weeks	IA$^+$/*n** (%)	Titers of IA$^+$, women with gestational diabetes (nIU/ml)	Months	IA$^+$/*n*† (%)	Titers of IA$^+$, women with gestational diabetes (nIU/ml)
1–3	2/50 (4)	88 ± 11	2	21/21 (100)	1,110 ± 940
4–6	3/44 (7)	367 ± 394	6	22/22 (100)	848 ± 986
7–9	14/32 (44)	613 ± 572	12	16/21 (76)	872 ± 933
10–12	12/19 (63)	897 ± 882	24	2/20 (10)	953 ± 914
13–16	7/13 (54)	1,291 ± 979			
>16	3/8 (38)	1,518 ± 39			

*Number of women with gestational diabetes still on treatment. †Number of previously IA$^+$ women with gestational diabetes available for follow-up.

The rate of adverse fetal outcome (defined as the presence of low Apgar score, hypoglycemia, hypocalcemia, hyperbilirrubinemia, polycythemia, respiratory distress, obstetric trauma, small or large for the gestational-age infant, or perinatal mortality) was compared in IA$^-$ and IA$^+$ mothers at the end of pregnancy. Additionally, we investigated the presence of insulin autoantibodies (IAAs)/IAs in 25 new-onset type I diabetic patients, before and at 3, 6, and 12 months after starting insulin therapy.

Insulin antibody assay

IAs and IAAs were measured in serum samples stored at −20°C. A competitive radiobinding assay, as described by Vardi et al. (12), was used for antibody detection. In this method, a supraphysiologic amount of cold HI (3 × 106 nIU/ml serum) for displacement and a long incubation period (7 days) are included to improve the sensitivity of the method.

The cutoff value for IA positivity in our laboratory (75 nIU/ml) was derived from the mean plus 3 SD (15 ± 20 nIU/ml) of a control group of 106 adults (normal distribution, pregnant and nonpregnant, no differences between both groups) (13). Nevertheless, the cutoff value for cord blood samples from nondiabetic pregnancies was higher: 131 nUI/ml (47 ± 28 nIU/ml). The need for a higher cutoff for cord-blood IA is supported by other authors (14–16); some of them speculate about "naturally occurring insulin antibodies in neonates" (15), even though this binding does not seem to be IgG mediated (16).

Our laboratory performance was assessed through participation in the Third Immunology and Diabetes Workshop (IDW) Insulin Autoantibody Proficiency Test Programme carried out in the period when the present study was done (sensitivity 71%, specificity 100%, and validity 82%).

Statistical analysis

Fisher's exact test, Student's *t* test for nonpaired observations, and single and multiple regression analysis were used when appropriate.

RESULTS — All but one woman with gestational diabetes were IAA negative at entry, but at the end of pregnancy, after insulin therapy, 22 of 50 (44%) became positive for IA. In addition, four women, whose late pregnancy IA determination was negative, shifted to positive 2 months postpartum.

Mean final IA titers in the whole group were 404 ± 715 nIU/ml (877 ± 879 in the IA$^+$ group and 32 ± 27 in the IA$^-$ group). Calculated rates of IA positivity during insulin treatment were highest at 10–12 weeks after insulin initiation (63%), and IA antibodies disappeared slowly 6 months after delivery (Table 1).

IAs were present in 4 of 25 (16%) type I diabetic patients at onset, in 92% after 3 months, and in 100% after 6 and 12 months on insulin therapy. Baseline IA titers (65 ± 216 nIU/ml) rose considerably at 3 months (1,068 ± 912 nIU/ml, $P < 0.01$), but titers at 6 and 12 months (1,388 ± 1,010 and 1,323 ± 948 nIU/ml, respectively) did not differ from those at 3 months. IA titers at 3 months in type I diabetic patients were higher than final titers in women with gestational diabetes. However, in the IA$^+$ subgroups, the difference did not reach significance (1,205 ± 932.5 in type I diabetes patients vs. 877 ± 879 nIU/ml in women with gestational diabetes, $P = 0.13$).

Mean insulin dose in women with gestational diabetes and type I diabetic patients was similar (0.44 ± 0.28 vs. 0.51 ± 0.27 IU · kg^{-1} · day^{-1}, NS), but treatment duration (9.3 ± 6.8 vs. 12.2 ± 1.9 weeks) was longer in type I diabetic patients at the time of IA measurement (Table 2).

In a multiple regression analysis where treatment duration (weeks) was included, IA titers at the end of pregnancy were only associated with the cumulative insulin dose ($r = 0.29$, $P < 0.05$). The relationship was mainly due to the 22 women who became IA$^+$ ($r = 0.42$, $P = 0.053$), since in the IA$^-$ group, no association was found (Fig. 1). However, cumulative insulin dose and treatment duration were closely associated ($r = 0.7$, $P < 0.01$). We found IA status concordance in all but one of the 15 maternal (M)-cord blood (C) sample pairs (8 M$^+$/C$^+$; 6 M$^-$/C$^-$; 1 M$^+$/C$^-$). Moreover, IA titers in cord blood were strongly related to those of the mothers ($r = 0.74$, $P < 0.01$) but not to treatment variables. The rate of adverse fetal outcome in newborns did not

Table 2—*IAA and IA response to insulin therapy in women with gestational diabetes and new-onset type I diabetic patients*

	Women with gestational diabetes	IDDM patients	*P* value
n	50	25	—
Baseline IAA (nIU/ml)	15.8 ± 20	64 ± 214	0.055
Baseline IAA$^+$ (%)	1 (2)	4 (16)	<0.05
IA after HI (nIU/ml)*	404 ± 715	1,068 ± 912	<0.001
IA$^+$ after HI (%)	26 (52)	23 (92)	<0.001
Age (years)	31.8 ± 4.3	19.7 ± 5.6	<0.0001
Weeks on HI	9.3 ± 6.8	12.1 ± 1.9	<0.05
Insulin dose (IU/Kg/d)†	0.44 ± 0.28	0.51 ± 0.27	NS

*At the end of pregnancy (women with gestational diabetes) or at 3 months (type I diabetic patients). †Mean insulin dose just before IA measurement.

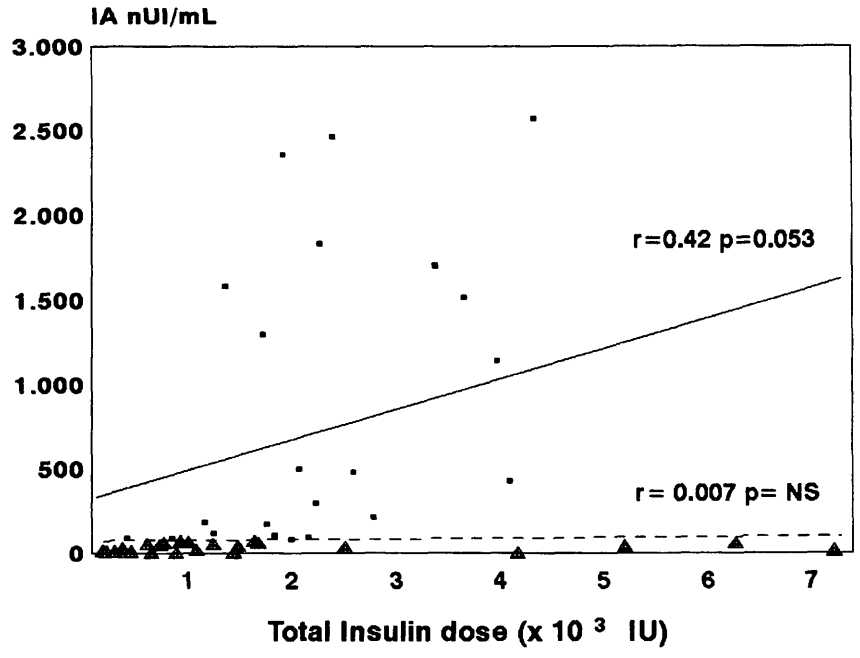

Figure 1—*Relationship between cumulated insulin dose and IA at the end of pregnancy in women with gestational diabetes who developed IA (■) and in those who did not (▲).*

differ according to the IA status of the mother at the end of pregnancy (27% in IA^-, 40% in IA^+, NS).

CONCLUSIONS — As could be expected, IAA positivity rate was higher in new-onset type I diabetic patients than in women with gestational diabetes. In the few reports that deal with this subject, the rate of IAA positivity in women with gestational diabetes seems to be similar to that of control subjects: 0% in both groups in the study of Damm et al. (17). However, in a previous study, we found an IAA positivity rate of 11% in the women with gestational diabetes islet cell antibody $(ICA)^+$ subgroup, which is comparable to the rate in ICA^+ type I diabetic relatives (13).

Despite treatment with HI, the rate of IA development after insulin therapy was high in both women with gestational diabetes and patients with type I diabetes. This is in agreement with previous reports and confirms that human insulin is immunogenic in both type I (18,19) and type II diabetes (20). The rate of women with gestational diabetes developing IAs at the end of pregnancy (44%) would be in agreement to the 60% described by Jovanovic et al. (6) of women with gestational diabetes injecting insulin with syringe (versus only 10% in those using a jet-injector). Expressed as percentage of insulin binding, mean final binding was 9% in IA^+ women with gestational diabetes and 14% in type I diabetic patients. Since insulin immunologically mediated adverse effects are mainly related to high titers (over 15–20% of insulin binding), the low-medium IA titers reached with HI may explain why IA-linked adverse reactions are infrequently observed with HI use (21,22). The additional finding of four IA^+ women at 2 months postpartum probably reflects the delay between the immunogenic stimulus and IA appearance. Another remarkable aspect is the long persistence of IAs after discontinuing insulin, and it is striking that at 2 years postpartum, two women with high titers at the end of pregnancy still were IA^+. It might be hypothesized that these women are developing type I diabetes (which does not seem probable, since their glucose tolerance 1 year after delivery was strictly normal) and that they are theoretically at risk of developing insulin allergy or insulin resistance, in the case that reintroduction of insulin is required.

The higher IA titers in new-onset type I diabetes could be due to a longer treatment period (only 26 of 50 women with gestational diabetes were on insulin therapy for >9 weeks), to insulin dose (the starting insulin dose was low [0.1–0.2 IU/kg/d] in women with gestational diabetes), or to intrinsic patient differences (20).

The concordance for IA status and the close relationship between IA titers in the maternal and cord blood samples support the IA transplacental passage from the mother to the fetus (23). The lack of relationship of IA status and fetal outcome can be due to the short exposure and to the aforementioned fact of IA being less important than glycemic control in influencing fetal outcome (11).

In summary, human insulin is immunogenic. Even in women with gestational diabetes receiving a short-term course, insulin therapy induces development of IA in about half of them. Although a IA^+ response may depend on individual factors, the cumulative insulin dose is slightly associated with the reached titers.

Acknowledgments — This work was supported by FISS (Fondo de Investigaciones Sanitarias de la Seguridad Social) grant 90/0412.

References

1. Chance RE, Root MA, Galloway JA: The immunogenicity of insulin preparations. *Acta Endocrinol* 83:185–195, 1976
2. Deckert T: The immunogenicity of new insulins. *Diabetes* 34 (Suppl. 2):94–96, 1985
3. Kurtz AB, Matthews JA, Mustaffa BE, Dagget PR, Nabarro JDN: Decrease of antibodies to insulin, proinsulin and contaminating hormones after changing treatment from conventional to purified pork insulin. *Diabetologia* 18:147–150, 1980
4. Finenberg SE, Galloway JA, Finenberg NS, Rathbun MJ: Immunologic improvement resulting from the transfer of animal insulin-treated diabetic subjects to human insulin (recombinant DNA). *Diabetes Care* 5 (Suppl. 2):107–113, 1982
5. Schernthaner G: Immunogenicity and allergenic potential of animal and human insulins. *Diabetes Care* 16 (Suppl. 3):155–165, 1993
6. Jovanovic L, Palmer J, Sparks S, Peterson CM: Jet-injected insulin is associated with decreased antibody production and postprandrial glucose variability when compared with needle-injected insulin in gestational diabetic women. *Diabetes Care* 16:1479–1484, 1993
7. Metzger BE, the Organizing Committee: Summary and Recommendations of the Third International Workshop-Conference on Gestational Diabetes Mellitus. *Diabetes* 40 (Suppl. 2):197–201, 1991
8. Langer O, Berkus M, Brustman L, Anyaegbunam A, Mazze R: Rationale for insulin management in gestational diabetes mellitus. *Diabetes* 40 (Suppl. 2):186–190, 1991
9. Mylgavanan R, Stowers JM, Steel JM, Wal-

lace J, MacHendry JC, Wright AD: Insulin immunogenicity in pregnancy: maternal and fetal studies. *Diabetologia* 24:19–25, 1983
10. Menon RK, Cohen RM, Sperling MA, Cutfield WS, Mimouni F, Khoury 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
11. Jovanovic L, Kitzmiller JL, Peterson CM: Randomized trial of human vs. animal species insulin in diabetic pregnant women: improved glycemic control, not fewer antibodies to insulin, influences birthweight. *Am J Obstet Gynecol* 167: 1325–1330, 1992
12. Vardi P, Dib SA, Tuttleman M, Connelly JE, Grinbergs M, Radizabeh A, Riley WS, MacLaren N, Eisenbarth GS, Soeldner JS: Competitive insulin antibody assay: prospective evaluation of subjects at high risk for development of type I diabetes mellitus. *Diabetes* 36:1286–1291, 1987
13. 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. *Diabetes Metab Rev* 12:275–285, 1996
14. Ziegler AG, Hillebrand B, Rabl W, Mayrhofer M, Hummei 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
15. Wellik SR, Veciana M, Morgan MA, Berkowitz KM, Arquilla ER: Naturally occurring insulin autoantibodies in neonates of normal pregnancies and their relationship to insulinemia and birth weight. *Am J Obstet Gynecol* 173:1878–1884, 1995
16. Castaño L, Bilbao JR, Calvo B, Urrutia I: Anti-insulin activity in new-born serum is not IgG-mediated (Abstract). *Diabetologia* 39 (Suppl. 1):96A, 1996
17. Damm P, Kühl C, Buschard K, Jakobsen BK, Svejgaard A, Sodoyez-Goffaux F, Shattock M, Botazzo GF, Molsted-Pedersen L: Prevalence and predictive value of islet cell antibodies and insulin antibodies in women with gestational diabetes. *Diabet Med* 11:558–563, 1994
18. Heding LG, Marshall MO, Persson B, Dahlquist G, Thalme B, Lindgren F, Akerblom HK, Rilva A, Knip M, Ludvigsson J, Stenhammar L, Strömberg L, Sovik O, Baevre H, Wofring K, Vidnes J, Kjaegard JJ, Bro P, Kaad PH: Immunogenicity of monocomponent human and porcine insulin in newly diagnosed type I (insulin-dependent) diabetic children. *Diabetologia* 27:96–98, 1984
19. Beaufort CE, Sodoyez JC, Koch M, Bruining GJ, Sodoyez-Goffaux F: Insulin autoantibodies and immune response to human insulin therapy in 24 type I (insulin-dependent) diabetic children: superiority of radiobinding assay over solid phase assay. *Diabetes Res Clin Pract* 21:19–24, 1993
20. Decochez K, Keymeulen, B, Van Gaal I, Gorus F, Somers G: Antibody response to exogenous human insulin in diabetes, preferential association with markers of autoimmunity? (Abstract). *Diabetologia* 39 (Suppl. 1):94A, 1996
21. Balsells M, Corcoy R, Lleonart R, Pujol R, Mauricio D, García AP, Pou JM, Cisteró A: Primary allergy to human insulin in a patient with gestational diabetes (Letter). *Diabetes Care* 14:423–424, 1991
22. Grammer LC, Roberts M, Buchanan TA, Fitzimons R, Metzger BE, Patterson R: Specificity of IgE and IgG against human insulin (recombinant-DNA) in human insulin allergy and resistance. *J Lab Clin Med* 109:141–146, 1987
23. Leiper JM, Finenberg SE, Lunan LB, Macluish AL: Insulin antibodies in the maternal and fetal circulation of pregnant diabetic women treated with human insulin of recombinant DNA origin. *Diabetes Res* 1:75–81, 1984