

# Insulin Antibody Responses After Long-Term Intraperitoneal Insulin Administration via Implantable Programmable Insulin Delivery Systems

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**OBJECTIVE** — To determine whether insulin antibodies are generated in diabetic patients after short- and long-term intraperitoneal insulin use and, if so, whether they are of potential clinical interest. Insulin antibodies commonly develop in diabetic patients who use subcutaneous human insulin, although their clinical significance remains controversial. Few data are available regarding insulin antibody responses to intraperitoneal insulin.

**RESEARCH DESIGN AND METHODS** — We studied insulin antibody levels and clinical diabetes control in 25 type 1 diabetic patients treated for 3–6 years with intraperitoneal surfactant-stabilized porcine modified human insulin delivered by implantable programmable insulin delivery systems.

**RESULTS** — All patients had preimplantation insulin antibody levels  $<20 \mu\text{U/ml}$ , with a mean value of  $2 \pm 2 \mu\text{U/ml}$  (1 SD). Mean antibody levels increased throughout the study period to a mean maximum of  $197 \pm 326 \mu\text{U/ml}$  ( $P < 0.02$ ) with 11 of 25 (44%) patients' levels exceeding  $20 \mu\text{U/ml}$  (insulin responders). The mean time to significant antibody development was  $21.8 \pm 4.4$  months. Of the 11 responder patients, 4 had clinical syndromes that consisted of increasing daily insulin requirements and/or nocturnal hypoglycemia despite minimal nighttime basal insulin infusion rates associated with peak antibody levels  $>200 \mu\text{U/ml}$ . None of the nonresponder patients (antibody levels  $<20 \mu\text{U/ml}$ ) had these clinical findings.

**CONCLUSIONS** — Our results indicate that insulin antibody levels observed during intraperitoneal administration of human insulin are 1) similar to those reported during subcutaneous administration; although the rise in antibody level may be delayed compared with subcutaneous human insulin, 2) associated with a patient subset who are insulin antibody responders after switching from subcutaneous to intraperitoneal human insulin, 3) associated with a decrease in levels among responder patients regardless of whether they discontinue or continue pump use, and 4) associated with increased insulin needs and/or nocturnal hypoglycemia despite minimal basal rate insulin infusion at nighttime when antibody levels exceed  $200 \mu\text{U/ml}$ .

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Type 1 diabetes, insulin-dependent diabetes mellitus; BA buffer, barbitol-albumin buffer; cpm, counts per minute; IgG, immunoglobulin-G; CV, coefficient of variation; ANOVA, analysis of variance; SPAD, subcutaneous peritoneal access device; HLA, human leukocyte antigen.

Antibodies to insulin commonly occur in diabetic patients both before and during insulin therapy. Antibody levels using beef-pork unpurified insulins ranged from 2,000–11,000  $\mu\text{U/ml}$  with an incidence of  $>90\%$  after one year of use (1–2). Frequency of responses and the circulating levels of these antibodies have significantly decreased because of the introduction of human insulin and with improvements in the purity of insulin preparations (3). Nevertheless, detectable levels of insulin antibody still occur after purified human insulin use in 29–85% of diabetic patients (1,4–6). Antibody levels in patients using only human insulin via subcutaneous injections range up to 757  $\mu\text{U/ml}$ , with 23% of patients having levels  $>50 \mu\text{U/ml}$  after one year of use (4,5). Whether human insulin antibody levels have clinical significance remains controversial (1,7–10). Clinical findings to date include insulin resistance, allergic reactions, lipodystrophy, microangiopathy, and altered insulin pharmacokinetics leading to elevated postprandial blood glucoses and delayed and/or prolonged hypoglycemia.

We have measured the level of insulin antibodies in a group of patients treated with intraperitoneal U100 and U400 insulins delivered via three implantable intraperitoneal insulin delivery systems. To evaluate whether intraperitoneal insulin preparations are antigenic, we have compared preimplantation antibody levels with those occurring after 0.5–6 years of implantable pump use. Our earlier short-term studies suggested no clinically significant rise in antibody levels (11). We now report a subset of patients who, when followed for longer periods of time, do generate measurable insulin antibody levels, similar to those observed in patients using subcutaneous human insulin. Further, we report an association between elevated levels of insulin antibodies and a clinical syndrome of increasing daily insulin needs and/or markedly decreased nighttime basal rate

insulin requirements coupled with nocturnal hypoglycemia.

## RESEARCH DESIGN AND

**METHODS**— We evaluated 25 male and 17 female adult type I diabetic (C-peptide negative) patients with a mean age of  $38 \pm 1.4$  years (range 25–63 years of age). All 42 individuals were evaluated as prospective pump patients, and they permitted a larger subset of data for prepump antibody level analysis. Their data are included for comparison with subcutaneous human insulin data described previously and in CONCLUSIONS. Of the patients, 25 were followed both before and after receiving an implantable insulin delivery system. The pump group's mean age was  $39 \pm 1.8$  years (range 25–63 years of age) and included 15 males and 10 females. No patients had significant diabetic complications, and all were using non-Hoechst insulins, i.e., either human Lilly, Novo, or Nordisk insulins at the preimplantation time. No preimplantation insulins contained Genapol. The study was approved by the University of California at Irvine Institutional Review Board, and informed consent was obtained from all patients.

Patients enrolled in the study received one or two of three types of implantable insulin pumps from two separate device manufacturers: PIMS and MIP (Minimed, Sylmar, CA) or Infusaid Model 1000 (Pfizer-Infusaid, Norwood, MA). Data presented in this study represent only patients evaluated at the University of California at Irvine. Eight patients received the PIMS pump. This was part of a two-center study. Of the original eight PIMS patients, four were eventually reimplanted with the MIP, and one additional patient has been added. This study is a multicenter trial involving 12 centers. Sixteen patients received the Infusaid model 1000. This study also is a multicenter trial involving four sites. The purpose of this study is to compare 1) insulin antibody levels for three different pumps, 2) U100 vs. U400 insulins, and

3) clinical syndromes using different pumps and insulins. No multicenter trial can address these comparisons. Surfactant-stabilized porcine modified human U400 insulin was used in the Minimed devices, and U100 was used in the Infusaid model (HOE 21 PH insulin, Hoechst AG, Frankfurt, Germany).

Insulin antibodies in the pump group are reported in all patients before implantation and then at 6-month or yearly intervals. Patients were evaluated for 0.5–6 years postimplantation. Not all patients had antibody level samples obtained at all time points, but all patients in the trial were evaluated on at least four separate occasions except two patients who stopped pump use before the completion of one year. Finally, not all patients have progressed to 72 months; thus fewer patients were evaluated at the later time points.

## Insulin antibody assay

Serum was tested for insulin antibodies using an equilibrium binding assay (12). Briefly, duplicate serum samples of these dilutions (100  $\mu$ l) were incubated for 72 h at 4°C with 100  $\mu$ l of barbital-albumin buffer (BA buffer), pH 7.5 (7 mM sodium barbital, 12 mM sodium acetate, 130 mM sodium chloride, and 0.5% bovine serum albumin), and 100  $\mu$ l (0.02 nM) of  $^{125}$ I-labeled human insulin (Amersham, Arlington Heights, IL). Of total counts per minute (cpm), 95–98% are precipitable by trichloroacetic acid. Duplicate samples of the same serum also were assessed for background or nonspecific binding by adding 100  $\mu$ l of nonradioactive monocomponent human insulin (30 nM) in BA buffer. This procedure eliminates potential nonspecific binding by other substances present in insulin preparations pre- and postimplantation. After incubation, 300  $\mu$ l of ice-cold carrier (0.36% bovine  $\gamma$ -globulin in BA buffer) and 600  $\mu$ l of ice-cold 30% (wt/vol) polyethylene glycol 6000 (Sigma, St. Louis, MO) in water were added. The precipitate was centrifuged at 1,000 g for 30 min at 4°C. The pellet was washed

with 1.0 ml ice-cold 15% polyethylene glycol and recentrifuged (13). The supernatants were decanted, and the pellets were assessed for radioactivity in a  $\gamma$ -counter. The results are expressed as microunits of insulin bound per milliliter of serum ( $\mu$ U/ml). The calculations of  $\mu$ U/ml are as follows.

1. Calculate for each test serum sample the percentage binding =

$$\frac{\text{precipitable cpm in sample} \times 100}{\text{total input cpm}}$$

2. Calculate for each sample the percentage nonspecific binding =

$$\frac{\text{precipitable cpm in sample with added nonradioactive insulin} \times 100}{\text{total input cpm}}$$

3. Calculate the percentage specific binding = test serum percentage binding – percentage nonspecific binding

4. For 100% specific binding, the equivalent picomolar concentration is 20. This calculation is as follows:

[ $^{125}$ I]tyrosine (A-14) human insulin 0.01 mCi, specific activity = 2,000 Ci/mmol on June 7, 1993. All samples were counted on May 24, 1993 (–14 days with correction factor of 1.1755). Therefore, the corrected specific activity on May 24, 1993 is 2,351 Ci/mmol; 0.01 mCi =  $4.254 \times 10^{-9}$  mmol =  $4.254 \times 10^{-12}$  mol. This amount of  $^{125}$ I was dissolved in 1.0 ml of buffer as stock solution. ( $4.254 \times 10^{-12}$  mol/ml stock solution.)

On the day of assay, 0.47  $\mu$ l of the stock was diluted to 10 ml with BA buffer to  $2.0 \times 10^{-15}$  mol/0.1 ml.

Each assay tube gets 0.1 ml or  $2.0 \times 10^{-15}$  mol [ $^{125}$ I]insulin.

If the sample specific binding is 100%, it is equivalent to  $2.0 \times 10^{-15}$  mol of insulin antibody/0.1 ml of the diluted serum sample, or 20.0 pM.

5. Calculate test sample serum insulin antibody level in microunits per milliliter of insulin bound per milliliter of serum =

Table 1—Insulin antibody before and after implantable pump treatment

Patient number	Preimplantation ( $\mu$ U/ml)	Postimplantation years ( $\mu$ U/ml)						
		0.5	1	2	3	4	5	6
1	1	1	3	—	1	0	2	0
2	3	2	4	4	5	23	66	48
3	4	5	9	—	86	11	3	41
4	6	16	19	280	1,021	521*	—	14
5	3	21	26	1,008	720	—	—	17
6	1	2	0.4	3	3	2	1	—
7	2	2	1	—	31	22		
8	0.3	10	17	—	103	38		
9	9	4	10	—	7	6		
10	1	5	1	—	17	1		
11	0.1	1	3	—	0	0		
12	4	107	143	—	146	65		
13	1	0.4	5	—	39	4*		
14	0.1	0.1	—	—	1			
15	1	4	—	—	8			
16	0.2	0	0	—	0			
17	4	—	0	1	0			
18	1	3	22	65	18			
19	6	—	55	205	84			
20	4	—	0.4	5	1			
21	1	—	0	1	0			
22	0.2	0	0.2	2				
23	3	98	345	593				
24	1	1	3					
25	4	5						
n	25	21	22	11	21	7	3	3
Quantiles (%)								
100 (maximum)	9	107	345	1,008	1,021	38	66	48
75	4	5	19	280	84	23	66	48
50 (median)	1	30	3	5	8	11	3	41
25	1	1	0.4	2	1	2	2	0
0	0.1	0	0	1	0	0	2	0

Data are microunits of insulin bound per milliliter of serum.

\*At month 42 of study.

$$\frac{\text{test sample \% specific binding} \times 20 \text{ pmole/} \\ \text{L} \times \text{dilution factor} \times 5,734 \times 25}{100 \times 1,000 \times 1,000}$$

(Insulin MW = 5,734; 25  $\mu$ U/ng)

To establish that the insulin-binding proteins measured in sera were immunoglobulin-G (IgG) insulin antibodies, we also precipitated the serum-binding proteins with goat antihuman IgG sera (Sigma). The assay was performed exactly as described above except

that 60  $\mu$ l of goat antihuman IgG plus 540  $\mu$ l 3% polyethylene glycol in BA buffer were used. Similar results were obtained from appropriately diluted serum samples from low, moderate, or markedly elevated levels of insulin antibody showing linearity of the assay. Also, acidification of the serum sample to remove bound antigen before the incubation step with [ $^{125}$ I]insulin did not affect the results of the assay for samples in the ranges of 8–60  $\mu$ U/ml. Thus, for sam-

ples assessed in the critical ranges near the upper normal range, it is not important to remove bound insulin. For each assay, a negative control (normal serum) and positive control (35  $\mu$ U/ml) were measured as external standards. The interassay coefficient of variation (CV) was 7.9% for antibody levels <10  $\mu$ U/ml and 19.4% for levels >20  $\mu$ U/ml, and the intra-assay CV was 2.6% for levels <10  $\mu$ U/ml and 3.2% for levels >20  $\mu$ U/ml. This assay measures heterogeneous insulin antibodies and has a sensitivity of 1.5  $\mu$ U/ml.

### Statistical analysis

All data are presented as means  $\pm$  1 SD unless otherwise stated. All insulin antibody data were transformed to natural logarithms (after the addition of one to each number) for each antibody level to normalize the distribution of values before statistical evaluations. All mean data shown in figures and tables are in microunits per milliliter and are derived from the natural logarithms. The 6-month and yearly periods were compared with the preimplantation levels using paired Student's *t* tests of the log-transformed data. Antibody levels at each time period were compared between responders and nonresponders using the unpaired Student's *t* test. For comparison across time separately within the nonresponder and responder groups, among the entire study group, and for comparisons between each group, the repeated measures analysis of variance (ANOVA) was used after natural log transformation. Comparisons of antibody levels between U100 and U400 groups were made using Wilcoxon's rank-sum test.

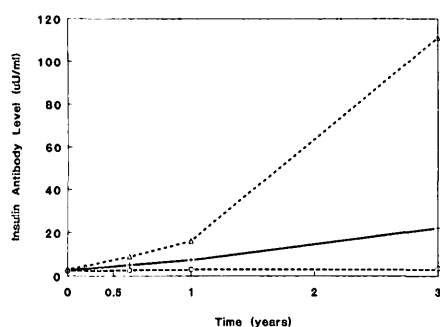
**RESULTS**—Those individual, mean, and median insulin antibody data for all patients are presented in Tables 1 and 2. The mean preimplantation insulin antibody level is  $2 \pm 2$   $\mu$ U/ml. In comparison with the preimplantation baseline, mean antibody levels in the total group increased throughout the first 3 years of study (Table 2 and Fig. 1). This progres-

Table 2—Implantable pump insulin

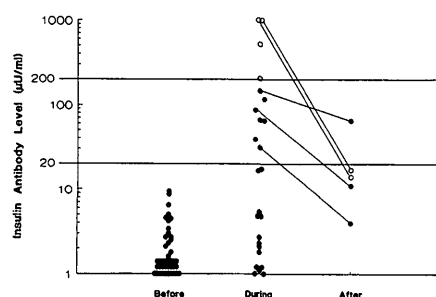
Year	n	Antibody levels (U-100)	
		Insulin antibody ( $\mu$ U/ml)	P value
Preimplantation	25	$2 \pm 2$	—
0.5	21	$14 \pm 30$	$<0.02$
1	22	$30 \pm 77$	$<0.02$
2	11	$197 \pm 326$	$<0.02$
3	21	$109 \pm 261$	$<0.002$
4	7	$15 \pm 14$	$<0.1$
5	3	$24 \pm 37$	$<0.4$
6	3	$30 \pm 26$	$<0.3$

Data are means  $\pm$  SD. P values determined with paired Student's t tests.

sive increase in mean antibody levels to  $197 \mu\text{U/ml}$  was caused by skewing of the total mean data by a subset of 11 of the 25 patients (44%). These patients were classified as insulin responders because their levels significantly exceeded the upper preimplantation range of  $20 \mu\text{U/ml}$  at 6 months and years 1–3 (Fig. 1). Fig-



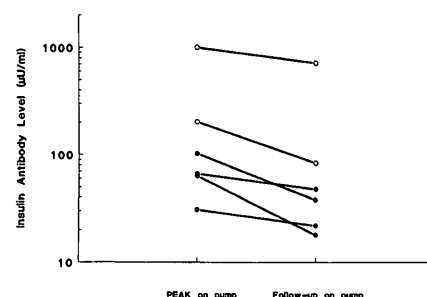
**Figure 1**—Mean insulin antibody levels in all 15 patients (+), nonresponder patients (O), and responder patients ( $\Delta$ ) before and during 3 years of follow-up. Repeated-measures ANOVA showed highly significant within-group antibody elevations in the total group and responder group compared with preimplantation ( $P < 0.0001$ ), whereas the nonresponder group showed no changes ( $P = 0.8$ ). Repeated-measures ANOVA also showed between group differences in the responder and nonresponder groups ( $P < 0.001$ ).



**Figure 2**—Individual insulin antibody levels before, peak values during, and levels after discontinuing implantable pump use. (O), Patients experiencing the clinical syndrome of nocturnal hypoglycemia despite decreased nighttime basal rates and/or increased total daily insulin needs. (●), Patients not experiencing such symptoms. The logarithmic scale is to the base 10; however, 20 and 200 are used to illustrate the normal and critically high levels for the associated clinical syndrome.

ure 1 shows the 3-year data for the 15-patient subset, where all data were available for preimplantation at 0.5, 1, and 3 years. From repeated measures ANOVA, the responder and total group had increased mean antibody levels, whereas the nonresponders did not when each group was evaluated across time. Further analyses showed that the responder group significantly differed from the nonresponder group (Fig. 1).

The individual preimplantation antibody levels can be compared with peak antibody levels in the responder and nonresponder subjects in Fig. 2. Responders included 3 of 8 (38%) patients using the PIMS device, 2 of 5 (40%) patients using the MIP device, and 6 of 16 (37.5%) patients using the Infusaid model 1000. Of the 11 responder patients, long-term follow-up showed insulin antibody levels to decrease in all 5 subjects who discontinued pump use and had follow-up antibody levels, and in 4 of these subjects, levels were  $<20 \mu\text{U/ml}$  (Fig. 2). Figure 3 shows the results of six responder patients remaining on implantable pump treatment. As shown, antibody levels decreased in all



**Figure 3**—Peak insulin antibody levels in individual patients and follow-up levels while remaining on implantable pump use longitudinally during the study. (O), Patients experiencing clinical syndromes. (●), Patients experiencing no clinical syndromes. A two-tailed paired Student's t test showed levels to decrease significantly at  $P < 0.01$ .

subjects, although intraperitoneal insulin treatment was continued.

To determine whether responder patients could be predicted based on preimplantation antibody levels, simple correlational analyses of pre versus peak levels were assessed. Correlation values for all patients were modest at  $r = 0.49$  ( $P < 0.013$ ) and  $r = 0.52$  and  $0.40$  for responders and nonresponders, respectively (NS for both).  $\chi^2$  analysis reveals that if preimplantation antibody levels are  $<1.5 \mu\text{U/ml}$ , the patient will likely remain  $<20 \mu\text{U/ml}$  (nonresponder) during pump use, and if the preimplantation level is  $>1.5 \mu\text{U/ml}$ , the patient will likely have levels  $>20 \mu\text{U/ml}$  (responder) during pump use ( $P < 0.05$ ). Thus by using two methods, predictability is suggested, but a larger number of patients will require study to determine whether the preimplantation antibody levels will be discriminative enough to be used clinically. A comparison with the patients using U400 and U100 insulins shows no differences in mean antibody levels when comparing the total groups (Table 3) or comparing the responders and nonresponders separately (data not shown).

From the longitudinal individual data (Table 1) and the mean data (Table

**Table 3—Antibody responses comparing U100 vs. U400 insulins**

Year	n	U100 insulin ( $\mu$ U/ml)	n	U400 insulin ( $\mu$ U/ml)
0	16	2 $\pm$ 0.6	9	3 $\pm$ 0.6
0.5	13	18 $\pm$ 10	8	7 $\pm$ 3
1	14	39 $\pm$ 25	8	15 $\pm$ 6
2	6	111 $\pm$ 89	5	300 $\pm$ 166
3	12	28 $\pm$ 13	7	274 $\pm$ 146
4	6	22 $\pm$ 10	4	9 $\pm$ 5

Data are means  $\pm$  SD.  $P = \text{NS}$ .

2), the mean time to significant antibody development, defined as  $>20 \mu\text{U/ml}$  (upper range preimplantation) was  $21.8 \pm 4.4$  months. Of the patients, 12% (3 of 25) developed significant antibody levels by 6 months postimplantation compared with 17% (4 of 24) after 1 year, 26% (6 of 23) after 2 years, and 50% (6 of 12) after 3 years. During 6 years of pump use, three patients have been followed of whom two are antibody responders (Table 1).

$\chi^2$  analysis from Wilcoxon's rank-sum analyses comparing antibody responder and nonresponder patients indicated no relationship of antibody generation to catheter occlusions or decreased insulin flow rates secondary to insulin aggregation in the pumping chamber. In fact, the trend was for antibody responder patients to have less pump insulin aggregations or catheter occlusions, because only 4 of 11 antibody pump responders had decreased flow rates or catheter problems, whereas 12 of 14 nonresponders had similar problems.

One patient (patient 19 in Table 1) in the MIP device trial had significantly elevated antibody levels before and during MIP use. Three years earlier he had used a subcutaneous peritoneal access device (SPAD) receiving intraperitoneal insulin through a reservoir requiring multiple daily injections of Regular insulin into the reservoir (14). For 2.5

years after SPAD use, his antibody levels were normal or near normal at 5, 6, and  $24 \mu\text{U/ml}$ . After MIP use, his antibody levels increased to a peak of  $205 \mu\text{U/ml}$  at month 24, but have subsequently decreased to  $84 \mu\text{U/ml}$  despite continued intraperitoneal insulin (see case 2 below).

To indirectly determine whether increased antibody levels were of potential clinical significance, we reviewed the clinical course of all patients. Of the 25 patients, 4 (16%, Figs. 2 and 3, open circles) had clinical syndromes that consisted of increasing daily insulin needs and/or nocturnal hypoglycemia (blood glucose  $<40 \text{ mg/dl}$ ), despite using minimal basal insulin rates and consuming snacks during the night. When the arbitrary antibody level of  $200 \mu\text{U/ml}$  is chosen, patients whose levels exceed this value (Fig. 2, open circles) are more likely to have the clinical syndrome, which is significantly different ( $P < 0.01$ ) from individuals in the  $20\text{--}200 \mu\text{U/ml}$  group (Fig. 2, closed circles).

### Case reports

**Case 1.** A 29-year-old female patient developed elevated antibody levels after 3 months of Infusaid pump use. She developed a peak antibody response of  $593 \mu\text{U/ml}$ , accompanied by a fivefold increase in total daily insulin requirements ( $40\text{--}60$  to  $200\text{--}300 \text{ U/day}$ ) to maintain daytime near-normal blood glucose levels and a decrease in nocturnal blood glucose ( $<40 \text{ mg/dl}$ ) despite a 10-fold decrease of her nighttime basal insulin rate from 5 to  $0.5 \text{ U/h}$ . This patient was explanted, but she and her primary physician have elected not to evaluate additional antibody samples.

**Case 2.** One 63-year-old male patient experienced a doubling of daily insulin requirements from 25 to  $52 \text{ U/day}$  following MIP pump use, along with repeated nocturnal hypoglycemia during a nighttime basal infusion rate of  $0.5 \text{ U/h}$  (peak antibody level =  $205 \mu\text{U/ml}$  at month 24). His antibody levels have decreased to  $84 \mu\text{U/ml}$  on intraperitoneal

insulin; he remains symptomatic but stable.

**Case 3.** A 25-year-old female patient developed increased antibody levels by 6 months postimplantation with the PIMS device, reaching a peak of  $1,008 \mu\text{U/ml}$ . The patient experienced repeated nocturnal hypoglycemia despite no nighttime insulin administration over 10 h, although total daily insulin requirements were unchanged at  $60\text{--}70 \text{ U/day}$ . She was explanted secondary to pregnancy, and before explantation her antibody levels decreased to  $720 \mu\text{U/ml}$ . After using subcutaneous human insulin for 3 years, her antibody levels are normal at  $17 \mu\text{U/ml}$ .

**Case 4.** One 26-year-old male patient developed elevated antibody levels after 20 months of PIMS pump use, reaching a peak level of  $1,021 \mu\text{U/ml}$ . He also experienced repeated nocturnal hypoglycemia despite no nighttime insulin infusion ( $0 \text{ U/h} \times 8 \text{ h}$ ), without any change in his total daily insulin requirements at  $90\text{--}100 \text{ U/day}$  ( $4.8 \text{ U/h} \times 16 \text{ h}$  plus  $15 \text{ U}$  premeal insulin). He was explanted because of metabolic instability and 6 months after explantation his antibody level decreased to  $521 \mu\text{U/ml}$ . After 3 years of subcutaneous human insulin, his antibody level is normal at  $14 \mu\text{U/ml}$ .

Other responder patients with peak antibody levels of 146, 103, 86, 66, 65, 39, and  $31 \mu\text{U/ml}$  did not have these clinical findings ( $P < 0.01$  vs. responders). None of the nonresponder patients had nocturnal hypoglycemia with low nighttime basal infusion rates of insulin. Only two severe hypoglycemic episodes occurred during the study period, both in the same antibody-negative patient.

**CONCLUSIONS**— This study reports insulin antibody levels in patients receiving intraperitoneal insulin that are similar to those reported in patients using subcutaneous human insulin (4–6,15). Unlike previous antibody studies of intraperitoneal insulin administration, we have identified insulin responder pa-

tients (44%) who develop an increase in insulin antibody levels above the preimplantation range after switching from subcutaneous human insulin to intraperitoneal human insulin. The antibody responses appear independent of the pump type, concentration of insulin, or catheter-related problems. A subset of these antibody responder patients (4 of 11 [16%]) also had a clinical syndrome comprising increased daily insulin requirements and/or nocturnal hypoglycemia despite minimal nighttime insulin infusion rates. Note that no serious acute or life-threatening sequelae were observed in these patients.

Although a parallel subcutaneous insulin treatment group was not evaluated in this trial, multiple insulin antibody studies are reported after subcutaneous insulin. In one key study, insulin antibodies were observed in 44% of new insulin use patients treated with only subcutaneous human insulin for one year (4). These levels ranged from 0–757  $\mu\text{U/ml}$ . At one year, another study reported a rate of 23% of patients with antibody levels  $>50 \mu\text{U/ml}$ , with levels ranging from 0–190  $\mu\text{U/ml}$  (5). Unpublished data in non-insulin-dependent diabetes mellitus patients treated with subcutaneous human insulin revealed a 54% incidence rate of antibody levels  $>50 \mu\text{U/ml}$  during 14 months of treatment, with 19% having levels  $>500 \mu\text{U/ml}$  (M. Zoltbrocki, unpublished observations) (16). Each of these studies used NPH and Regular insulins rather than surfactant stabilized pump insulin. Although insulin preparations vary from our intraperitoneal insulin study and prior subcutaneous insulin studies, our findings include a similar range for insulin antibody levels, albeit at a somewhat slower rate of antibody development, i.e., 17% incidence after one year vs. 23, 38, and 44% in previous studies (4,5). No long-term reports use continuous subcutaneous insulin infusion or intraperitoneal insulin during intraperitoneal dialysis in renal failure.

Few data have been published re-

garding antibody levels in patients treated with intraperitoneal human insulin. Published studies have reported detectable insulin antibody levels in 30 and 100% of patients before implantation, with no change in the percentage of antibody positive patients or in the level of insulin antibody (peak value  $>700 \mu\text{U/ml}$ ) after 12–18 months of intraperitoneal insulin use (11,17,18). In more recent short-term preliminary reports, an increase in insulin antibody levels also was observed in patients using both U100 and U400 insulins (19–22). In one of these reports, short-term (2 months) intraperitoneal insulin caused a transient sixfold increase in antibody levels (19). Previously, we have described similar cellular transient immune changes soon after subcutaneous human insulin use (23). Our data reported here indicate that patients who are antibody responders have responses that are relatively long lasting, at least  $>1$  year, and that regardless of whether patients discontinue or continue pump use the antibody levels decrease.

It is unclear why certain patients are antibody responders and why only some of these responders have the associated clinical syndromes we describe. Several factors have been identified previously that may influence the production of insulin antibodies. Human leukocyte antigen (HLA)-DR4 and HLA-B15 have been linked with a high insulin antibody response, whereas HLA-DR3 and HLA-B8 are associated with less frequent antibody responses (3,5,8). Insulin purity, species and formulation, along with a history of intermittent insulin use also have been associated with the development of insulin antibodies (3). It is possible that 1) surfactant, 2) intraperitoneal placement of insulin, and/or 3) insulin aggregates could be factors determining which patients become antibody responders. The presence of insulin monomers versus aggregates may be important, with loss of tolerance developing in certain patients. The finding that protein aggregates can diminish the tol-

erance induced by monomers is well described in mice (24).

The following observations suggest that the site of insulin administration is important for antibody development: 1) short-term (one year) subcutaneous human insulin results in increased antibody levels (as high as 700  $\mu\text{U/ml}$ ) in roughly 50% of patients, 2) because insulin antibody levels are virtually always  $<20 \mu\text{U/ml}$  before intraperitoneal insulin use in our total series ( $n = 86$ ), antibodies to subcutaneous human insulin must have a spontaneous drop to  $<20 \mu\text{U/ml}$ , and thus 3) the new insulin delivery site (intraperitoneal) may reactivate the immune system in these responder patients, albeit more slowly than with subcutaneous insulin.

Of the 11 antibody responders in our study, 4 had an associated syndrome of increased daily insulin requirements and/or nocturnal hypoglycemia despite low basal nighttime infusion rates. Other preliminary reports confirm the observations of clinical changes such as nighttime hypoglycemia and lower fasting blood glucose in patients with elevated antibody levels (20,21). None of the nonresponders in our series developed this syndrome. This clinical syndrome may be related to altered insulin pharmacokinetics as described previously in patients with high insulin antibody levels during subcutaneous insulin use (3). In our patients, this syndrome is statistically associated with antibody levels  $>200 \mu\text{U/ml}$ . These high antibody levels also were associated with pump removal in two patients. The pharmacokinetic argument suggests that patients having antibody levels  $<200 \mu\text{U/ml}$  also could develop the syndrome, but larger patient groups may need to be evaluated. High insulin antibody levels also have been associated with other clinical entities, including lipodystrophy and immunological insulin resistance (3). The nocturnal hypoglycemic syndrome we describe is not clearly causal, but indirectly associated

with elevated levels of insulin antibodies, and this issue could be pursued using more direct methods in future patients.

We conclude that insulin antibody formation occurs in some patients treated with long-term intraperitoneal human insulin via implantable insulin devices, which reach absolute antibody levels and frequency rates similar to those reported for subcutaneous human insulin use. Although surfactant may possibly enhance this response, this is unlikely because the results of antibody levels and frequency rates are similar to nonsurfactant containing human insulins. The rise in antibody levels, though, is delayed compared with most reports of subcutaneous human insulin use. Because our patients using subcutaneous human insulin all had preimplantation antibody levels  $<20 \mu\text{U/ml}$ , it appears that this new site of insulin administration may reactivate the immune system. When markedly elevated, insulin antibodies may be associated with increased insulin needs and/or nocturnal hypoglycemia despite a minimal nighttime basal rate of insulin. A cause-and-effect relationship, however, has not been proven. Also undetermined is what factors are involved in determining which patients become antibody responders and what causes some of these responders to develop this clinical syndrome.

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## References

- Heding LG, Larsson Y, Ludvigsson J: The immunogenicity of insulin preparation: antibody levels before and after transfer to highly purified porcine insulin. *Diabetologia* 19:511–15, 1980
- Fineberg SE, Galloway JA, Fineberg NS, Goldman J: Effects of species of origin, purification, levels and formulation of insulin immunogenicity. *Diabetes* 32:592–99, 1983
- Van Haeften TW: Clinical significance of insulin antibodies in insulin-treated diabetic patients. *Diabetes Care* 12:641–48, 1989
- Fineberg SE, Galloway JA, Fineberg NS, Rathbun MJ, Hufferd S: Immunogenicity of recombinant DNA human insulin. *Diabetologia* 25:465–69, 1983
- Scherthaner G, Berkenstein M, Fink M, Mayr WR, Menzel J, Schober E: Immunogenicity of human insulin (Novo) or pork monocomponent insulin in HLA-DR-typed insulin-dependent diabetic individuals. *Diabetes Care* 6 (Suppl. 1):43–48, 1983
- Velcovsky HG, Federlin KF: Insulin-specific IgG and IgE antibody response in type I diabetic subjects exclusively treated with human insulin (recombinant DNA). *Diabetes Care* 5:126–28, 1982
- Sklenar I, Wilkin TJ, Diaz JL, Erb P, Keller U: Spontaneous hypoglycemia associated with autoimmunity specific to human insulin. *Diabetes Care* 10:152–59, 1987
- Scherthaner G, Ludwig H, Mayr WR: Immunglobulin-G insulin antibodies and immune region-associated alloantigens in insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 48:403–407, 1979
- Waldhausl WK, Bratusch-Marrain P, Kruse V, Jensen I, Nowotny P, Vierhapper H: Effect of insulin antibodies on insulin pharmacokinetics and glucose utilization in insulin-dependent diabetic patients. *Diabetes* 34:166–73, 1985
- Van Haeften TW, Heiling VJ, Gerich JE: Adverse effects of insulin antibodies on postprandial plasma glucose and insulin profiles in diabetic patients without immune insulin resistance. *Diabetes* 36:305–309, 1987
- Sauadek CD, Selam JL, Pitt HA, Waxman KW, Rubio M, Jeandidier N, Turner D, Fischell RE, Charles MA: A preliminary trial of the programmable implantable medication system for insulin delivery. *N Engl J Med* 321:574–79, 1989
- Goldman J, Baldwin D, Pugh W, Rubenstein AH: Equilibrium binding assay and kinetic characterization of insulin antibodies. *Diabetes* 27:653–60, 1978
- Desbuquois B, Aurbach GD: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J Clin Endocrinol Metab* 33:732–38, 1971
- Stephan RL, Greggoni D, Hanover BK: Intraperitoneal biocompatible implants. In *Infusion Systems in Medicine*. Ensminger WD, Selam JL, Eds. Mount Kisco, New York, Futura, 1987, p. 47–52
- Iavicoli M, di Mario U, Coronel GA: Semisynthetic human insulin: biologic and immunologic activity in newly treated diabetic subjects during a 6-month follow-up. *Diabetes Care* 7:128–31, 1984
- Christiansen AH: A new method for determination of insulin-binding immunoglobulins in insulin-treated diabetic patients. *Horm Metab Res* 2:187–88, 1970
- Point Study Group: One-year trial of a remote-controlled implantable insulin infusion system in type I diabetic patients. *Lancet* 2:866–69, 1988
- Wredling R, Adamson U, Lins PE, Backman L, Lundgren D: Experience of long-term intraperitoneal insulin treatment using a new percutaneous access device. *Diabetic Med* 8:597–600, 1991
- Jeandidier N, Rosart-Ortega F, Keipes M, Lory S, Hauptmann G, Pinget M: Immunogenicity of long-term implantable insulin infusion using two different programmable pumps: arguments for multiple factors involved in the increase of insulin antibodies (Abstract). In *Artificial Insulin Delivery Systems, Pancreas and Islet Transplantation*. Berlin, Springer-Verlag, 1992, p. 37
- Boivin S, Jeandidier N, Sapin R, Réville P, Pinget M: Immunogenicity of long-term intraperitoneal insulin infusion using implantable pumps, high anti-insulin antibodies (AIA) levels clinical consequences (Abstract). *Horm Metab Res* 25:51, 1993
- Georges LP, O'Brian JT, Davidson PC, Thornton KR, Edwin S, Fineberg NS, The MiniMed Pump Investigators: Intraperitoneal delivery of U400 human insulin is immunogenic. *Diabetes* 42 (Suppl. 2):183A, 1993
- Lassmann-Vague V, Belicar P, Raccach D, Vialettes B, Lassmann-Vague PhV: Immunogenicity of long-term intraperitoneal insulin infusion and relation to metabolic

- control. *Diabetologia* 36 (Suppl. 1):A38, 1993
23. Eichner HL, Lauritano AA, Woertz LL, Selam JL, Gupta S, Charles MA: Cellular immune alterations associated with human insulin therapy. *Diabetes Res Clin Pract* 8:111–15, 1988
24. Gahring LC, Weigle WO: The induction of peripheral T-cell unresponsiveness in adult mice by monomeric human V-globulin. *J Immunol* 143:2094–2100, 1989