【 CASE REPORT 】

Recurrent Hypoglycemia Due to a High Titer of Insulin Antibody in Response to Exogenous Insulin Administration in Two Cases of Type 1 Diabetes

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Abstract:

In the first case, a 60-year-old man who was using continuous subcutaneous insulin infusion (CSII), developed recurrent hypoglycemia due to insulin antibodies. This is the first report of such a case using CSII. In the second case, a 70-year-old man was follow-up case who developed hypoglycemia while using human insulin. In both cases, the hypoglycemia subsided after switching to multiple daily insulin injection and/or insulin preparation. The results of Scatchard analyses of the two cases were similar to those of cases of insulin autoimmune syndrome (IAS) that improved after recovery from hypoglycemia. The clinical characteristics and Scatchard analysis data were essentially the same as those for IAS, except for the presence of insulin administration.

Key words: insulin administration, insulin antibody (IAb), hypoglycemia, continuous subcutaneous insulin infusion (CSII), Scatchard analysis, insulin autoimmune syndrome (IAS)

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Introduction

Insulin administration is accompanied by various types of immune responses to insulin, including the production of an insulin antibody (IAb), the development of an insulin allergy or lipoatrophy. With the advent of highly purified recombinant human insulin or human insulin analogs, these complications have dramatically decreased, but they still occur on occasion. Studies of large populations have failed to establish a relationship between IAb and the rates of hypoglycemic events or glycemic control (1), but sporadic cases of severe hypoglycemia continue to be reported.

Fineberg et al. reviewed two types of rare syndromes involving recurrent or prolonged hypoglycemia due to the production of IAb (2). The first is insulin autoimmune syndrome (IAS), which is characterized by the spontaneous development of hypoglycemia without exogenous insulin administration and the presence of a high titer of insulin autoantibodies (IAA) (3). IAS was first reported by Hirata et al. in 1970 and is thus referred to as Hirata's disease (4). Since then, hundreds of cases of IAS have been reported, nearly exclusively in Japan but sometimes in East Asia as well. Scatchard analyses of IAA show characteristic features including a low affinity (K1) and a large capacity (B1) at a high-affinity, low-capacity population (5). Furthermore, Uchigata et al. reported a strong association of IAS with human leukocyte antigen (HLA) DRB1*04:06 (6).

The second hypoglycemic syndrome is caused by a high titer of IAb in response to exogenous insulin administration.

In 1960, Harwood reported a woman with type 1 diabetes mellitus (T1DM) who developed recurrent or prolonged hypoglycemia due to the formation of IAb to exogenously administered insulin (7). Although highly purified human or analog insulin is currently in use, as described above, hypoglycemic cases have been sporadically reported (exclusively in Japan) (2, 8-12) and appear to be similar to the cases of IAS. Interestingly, Scatchard analyses of IAb in previously reported cases have shown characteristics similar to those of IAS.

However, some differences have been reported between the hypoglycemic cases and cases of IAS (3). First, the patients with hypoglycemia had received exogenous insulin, but not those with IAS. Most IAS patients have HLA DRB1 *04:06, but not the patients with hypoglycemia. A causal relationship with sulfhydryl (SH) drugs has also been reported for IAS but not for the hypoglycemic cases. Spontaneous remission occurs in about 82% of IAS patients but not in the hypoglycemic cases.

We herein report two patients with T1DM who developed recurrent hypoglycemia caused by a high titer of IAb in response to exogenous insulin administration. The first patient was using continuous subcutaneous insulin infusion (CSII) and developed recurrent hypoglycemia. The second patient is a follow-up case of a subject who developed recurrent hypoglycemia, which then subsided spontaneously despite the fact that the same insulin preparation was still being used.

Case Reports

A Scatchard analysis using human insulin

Serum that had been deinsulinized by treatment with dextran-coated charcoal was used for the Scatchard analysis (13). The serum was incubated with 100 μL of ^{125}I-human insulin (1.2×10^4 cpm/30-40 pg of human insulin/tube) in the presence of 100 μL of serial concentrations of a human insulin solution. After precipitation with polyethylene glycol (PEG) 6000, the radioactivity of the pellet was counted with an automatic gamma counter. The assay was measured according to the method by Eguchi et al. (5, 14) (SRL, Tokyo, Japan).

Regarding Case 2, since the assay at the onset was performed using pig insulin as described in previous report (15), we reperformed the assay using human insulin with the serum stocked in a deep freezer at the onset.

Case 1

In June 2012, a 60-year-old man complained of thirst, polyuria and body weight loss and visited a hospital. He was diagnosed with diabetes and treated with glimepiride 1 mg. He simultaneously started on a carbohydrate-restriction diet by himself. In September, his blood glucose control improved, and glimepiride was stopped. One year later he was referred to our hospital due to poor glycemic control. At the time of the first admission in March 2013, his height, body weight and body mass index (BMI) were 169 cm, 54.2 kg and 18.8 kg/m^2, respectively. His fasting blood glucose 281 mg/dL, HbA1c 9.9%, urinary ketone body 3+, fasting serum C-peptide 0.5 ng/mL and glutamic acid decarboxylase (GAD) antibody (Ab) 4,030 U/mL were compatible with the diagnosis of slowly progressive insulin-dependent diabetes mellitus (SPIDDM). He started CSII (Paradigm insulin pump MMT-722; Medtronic, Northridge, USA) using a human insulin analog (lispro; Eli Lilly, Indianapolis, USA) with carbohydrate counting, which improved his blood glucose levels, as shown in Fig. 1a. Since he was able to manage CSII as well as carbohydrate counting by himself, his HbA1c levels remained acceptable.

One and a half years later, in late October 2014, he developed nocturnal hypoglycemia and daytime hyperglycemia without any obvious cause that continued despite decreasing the basal infusion rate of CSII and frequently ingesting carbohydrates before bedtime (Fig. 1b, c). Since frequent nocturnal hypoglycemia disturbed his sleep pattern, he was readmitted to our hospital in November 2014.

At the time of his second admission, his body weight and BMI were 63.5 kg and 22.2 kg/m^2, respectively (Table 1). A physical examination and his medical history and family history of diabetes were unremarkable. He had no diabetic microvascular complications, such as nephropathy, neuropathy or retinopathy. Blood glucose and serum C-peptide levels at 2 hours after a meal were 229 mg/dL and 0.7 ng/mL respectively. HbA1c was 6.9%, and GAD Ab was still positive at 1,285 U/mL (RIA). Renal and hepatic function tests were almost within the normal ranges, and counter regulatory hormones against insulin action had not been reduced (Table 2). Neither computed tomography nor magnetic resonance imaging revealed any tumors in the pancreas. These findings suggest that the hypoglycemia might have been caused by an IAS-like IAb, as reported previously.

We detected a high titer of IAb (88.7%) (Table 1). A Scatchard analysis of the IAb showed an extremely lower affinity constant (K1) and a higher binding capacity (B1) at a high-affinity, low-capacity population with the same characteristics as that of IAS (5) (Fig. 2a, Table 3). These data suggested the IAb might have caused the nocturnal hypoglycemia and daytime hyperglycemia, as described in previous cases of IAS (5).

We initially recommended steroid treatment, but this was not acceptable for the patient because of possible side effects. We then decreased the basal insulin infusion rate at night to the lowest level of 0.05 U/min and simultaneously changed his human insulin analog to another one (glulisine; Sanofi, Paris, France), but this failed to improve the nocturnal hypoglycemia (Fig. 1d, left panel). Since CSII, MMT-722 does not have a function to stop basal insulin infusion, we changed CSII to multiple daily insulin injections (MDI) using the same insulin analog three times before each meal without bedtime insulin (Fig. 1d, right panel). The insulin doses were simultaneously adjusted using carbohydrate counting and taking frequent bedtime snacks, and an α-
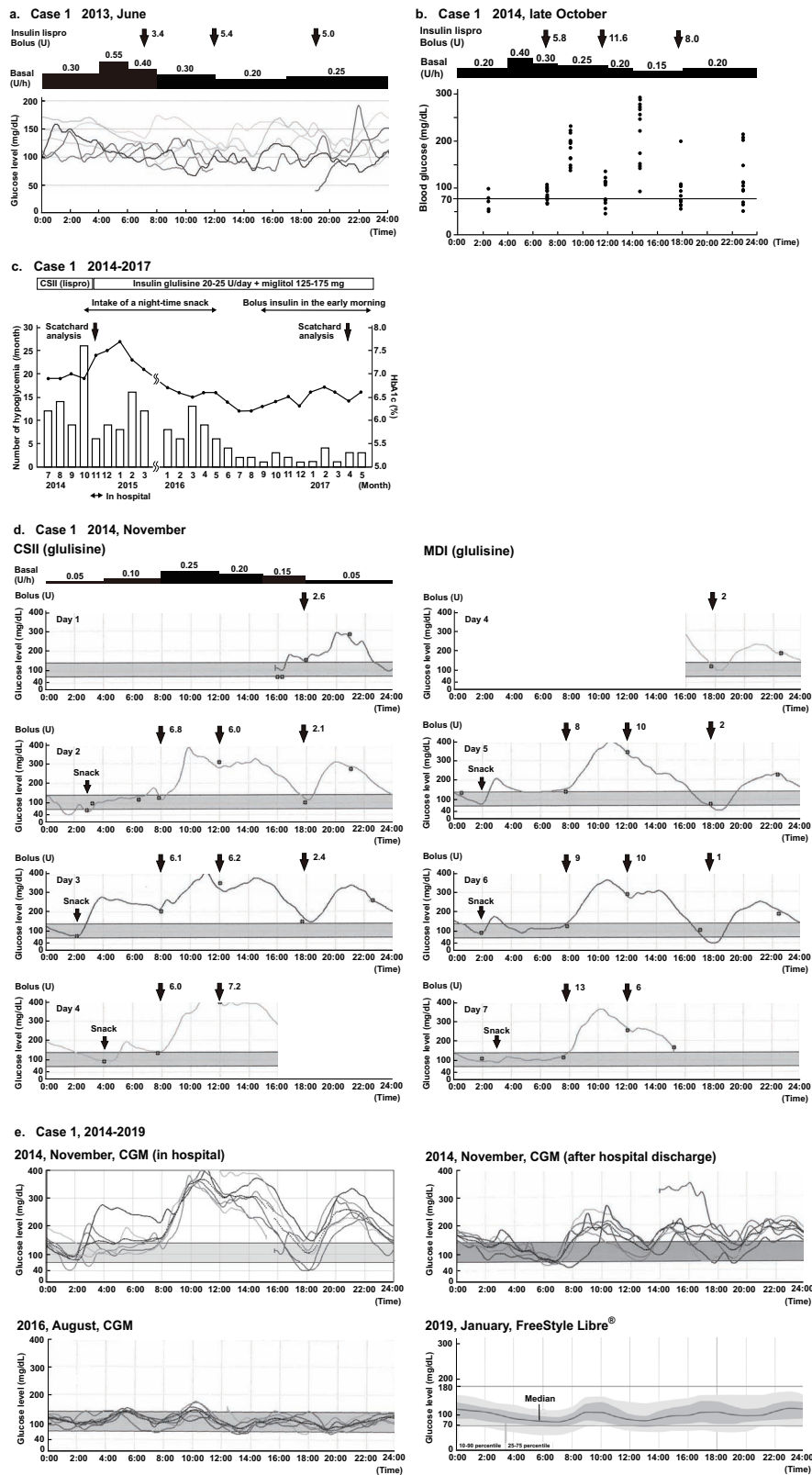
Figure 1. **a: Blood glucose profile with CGM and daily insulin doses with CSII after the first admission (2013, June). b: Blood glucose profile with SMBG and daily insulin doses with CSII before the second admission (2014, late October). c: Profile of the clinical course from 2014 to 2017. Numbers of hypoglycemia and the HbA1c levels are shown as bar and line graphs, respectively. Hypoglycemia was counted by the SMBG records from the patient (below under 70 mg/dL). d: Blood glucose profile with CGM and daily insulin doses for 6 days at the second admission (2014, November). The left panel shows CSII for the first three days, during which lispro was changed to glulisine, and the right panel shows MDI for the next three days with glulisine before each meal. e: Blood glucose profile with CGM (iPro2; Medtronic or FreeStyle Libre; Abbott, USA) from 2014 to 2019.**

Table 1. Clinical Characteristics, Laboratory and Genetic Findings at the Onset of and after the Treatment of Hypoglycemia.

	Case 1		Case 2	
	At onset	After treatment	At onset†	After treatment
Year	2014, November	2017	2005	2011
Age	63	66	70	76
BMI	22.2	21.1	17.1	21.8
HbA1c (%)	6.9	6.6	12	8.8
CPR, 2 h after meal (ng/mL)	0.7	0.1	0.2	<0.03
Insulin preparation	Lispro (CSII)	Glulisine (MDI)	Humacart R, Novolin N	Aspart (MDI)
Insulin dose (U/day)	30.1	32	28	22
Insulin antibody (%)	88.7	82.9	89	78.8
GAD Ab (U/mL)*	1,285	>2,000	-	-
IA-2 Ab (U/mL)	nd	0.8	-	-
Insulin allergy	-	-	+	-
Insulin lipoatrophy	-	-	-	+
HLA DR-DQ genotype	DRB1*09:01-DQB1*03:03/		DRB1*04:05-DQB1*04:01/	
	DRB1*13:02-DQB1*06:04		DRB1*09:01-DQB1*03:03	
Insulin gene genotype (class)	I/I		I/I	
BACH2 rs3757247 genotype‡	T/T		T/T	

CPR: C-peptide, GAD Ab: anti-glutamic acid decarboxylase antibody, IA-2 Ab: anti-insulinoma-associated antigen-2 antibody, CSII: continuous subcutaneous insulin infusion, MDI: multiple daily insulin injections, nd: not determined

*GAD Ab was assayed using radio immunoprecipitation assay (RIA) in 2005, 2011, and 2014 and enzyme immunoassay (EIA) in 2017.

†As described in reference 15. ‡As described in reference 48.

Table 2. Laboratory Findings at Onset (Case 1).

WBC (/μL)	6,800	ACTH (pg/mL)	29.4
Neut (%)	46.0	Cortisol (μg/dL)	18.0
Lymph (%)	42.6	Adrenaline (ng/mL)	0.28
Mono (%)	4.4	Noradrenaline (ng/mL)	0.75
Eosino (%)	6.6	Dopamine (ng/mL)	<0.01
Hb (g/dL)	15.0	TSH (μIU/mL)	0.623
Plt (×10⁴/μL)	20.6	FT4 (ng/dL)	1.37
T-Bil (mg/dL)	1.2	FT3 (pg/mL)	2.54
AST (IU/L)	39	GH (ng/mL)	0.25
ALT (IU/L)	46	IGF-1 (ng/mL)	61
LDH (IU/L)	169		
ALP (IU/L)	188	Urine glucose	3+
γ-GTP (IU/L)	46	ketone	-
BUN (mg/dL)	15.6	protein	-
Cre (mg/dL)	0.9	blood	-
eGFR (mL/min/1.73m²)	68.1		
UA (mg/dL)	4.5		
T-Chol (mg/dL)	231		
HDL-C (mg/dL)	99		
LDL-C (mg/dL)	108		
TG (mg/dL)	123		
AMY (U/L)	114		

glucosidase inhibitor was added three times to both preparations. These preparations improved the nocturnal hypoglycemia as well as the daytime hyperglycemia one week after discharge (Fig. 1e).

In 2016, the patient noticed there was no need for a bedtime snack to prevent nocturnal hypoglycemia and started small doses of the same insulin analog in the early morning to prevent morning hyperglycemia; at the end of 2017, we added insulin degludec (Novo Nordisk, Bagsvaerd, Denmark) in the evening as basal insulin instead of glulisine in the early morning to prevent early-morning hyperglycemia. These practices further improved his blood glucose profile (Fig. 1e). In 2017, his high IAb titer was slightly decreased to 82.9% (Table 1). GAD Ab and IA-2 Ab were both positive, and the serum C-peptide levels were decreased, indicating a continuing autoimmune process. The Scatchard analysis data were greatly improved; a higher binding capacity (B 1) was greatly decreased to almost one-tenth of that at the onset, in contrast to only a slight improvement in an extremely lower affinity constant (K1) (Fig. 2b).

These results suggest that the changes in the Scatchard analysis data correspond well to the changes in hypoglycemic attacks, as described by Eguchi et al. (14). The IAb titer was gradually decreased to 62.1% in 2018 and 35.1% in 2021.

Case 2

The second case is one of the three or six cases of insulin-triggered T1DM, at the onset, parts of which have been previously reported (15, 16). In brief, a 70-year-old man was referred to Ehime University Hospital with brittle diabetes and an insulin allergy. In January 2005, he was hospitalized elsewhere due to the development of facial palsy and diagnosed with type 2 diabetes, at which point he started human insulin (Humacart R; Eli Lilly) (Fig. 3a). After his blood glucose control had improved, his insulin was
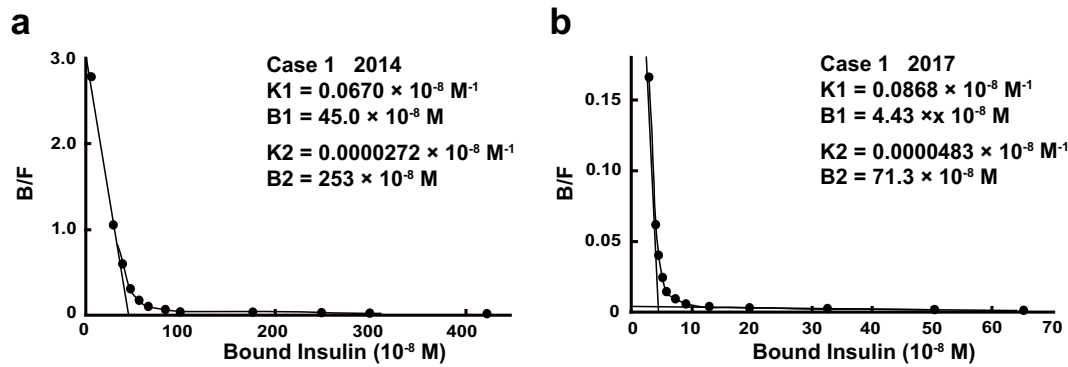
Figure 2. a: Scatchard analysis at the onset (before the treatment) of hypoglycemia (2014). b: Scatchard analysis after the treatment for hypoglycemia (2017).

Table 3. Scatchard Analysis and Insulin Antibodies in the IAS Cases and Control Insulin-treated Diabetics (5).

|	K1 ($\times 10^8$ M^{-1})	B1 ($\times 10^{-8}$ M)	Insulin antibody (%)
IAS (n=4)	0.138±0.0511	23.7±9.87	77.8±4.16
Control insulin-treated diabetics (n=5)	3.14±1.10	0.376±0.189	61.6±3.70

Values are given as the mean±SE.
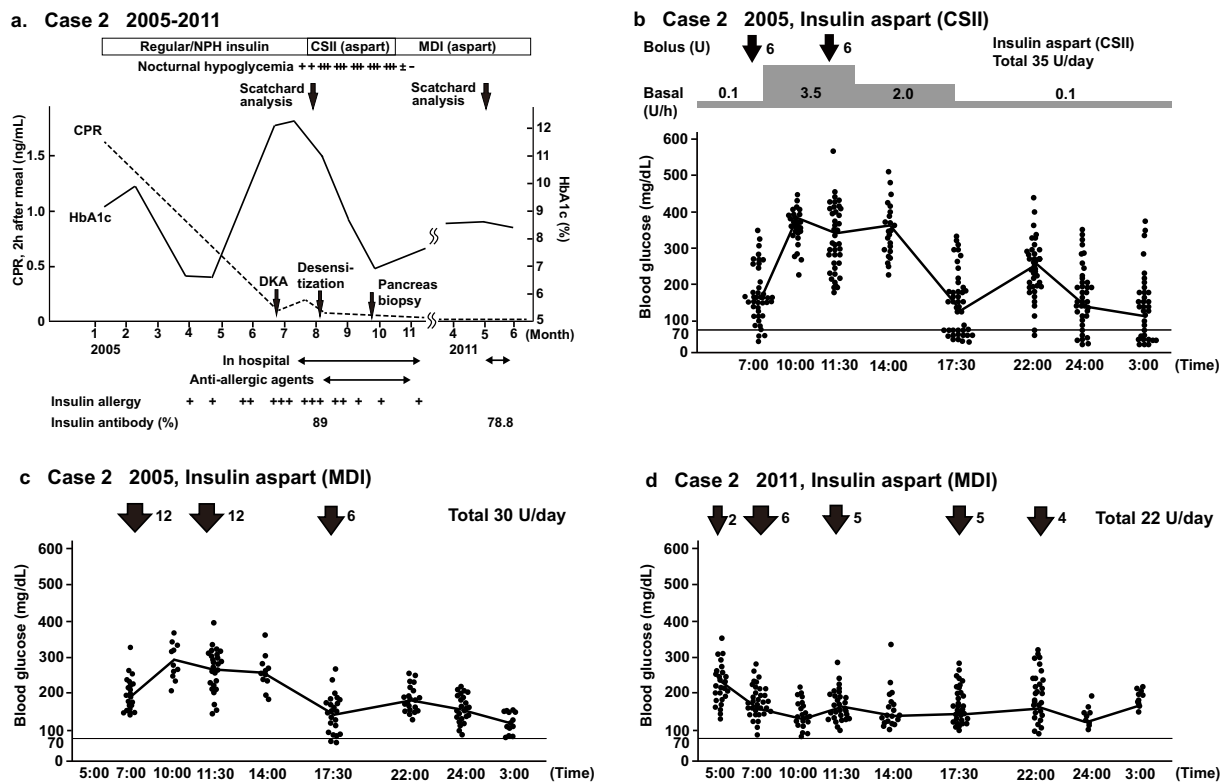
Figure 3. a: Profile of the clinical course from 2005 to 2011. The onset of hypoglycemia or insulin allergy or DKA is shown. b: Blood glucose profile and daily insulin doses of aspart with CSII at the first admission (2005). c: Blood glucose profile and daily insulin doses of aspart with MDI before each meal at the first admission (2005). d: Blood glucose profile and daily insulin doses with MDI after adjusting the insulin doses during the second admission (2011).

changed to Novolin N (Novo Nordisk), and he was discharged. At the end of March, he noticed an insulin allergy at the insulin injection sites. In April, he complained of thirst and polyuria, and his HbA1c levels were acutely elevated. In June, he had general malaise and nausea and was transferred to a hospital due to diabetic ketoacidosis (DKA).
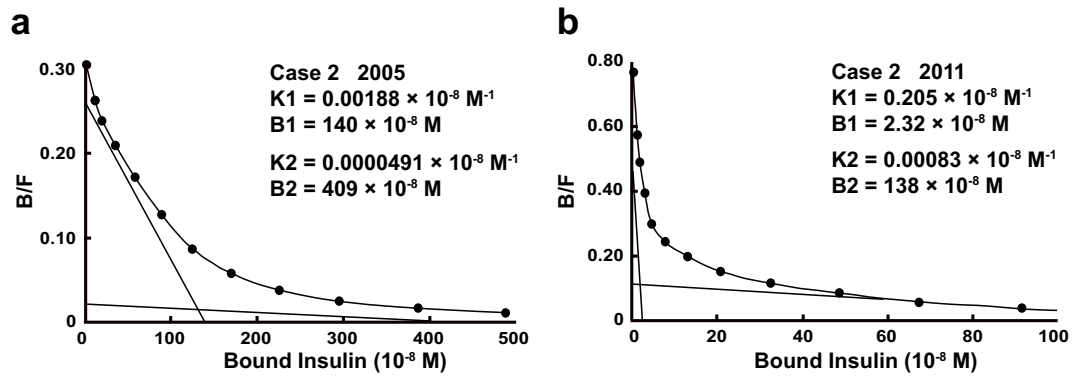
Figure 4. a: Scatchard analysis at the onset (before the treatment) of hypoglycemia (2005). b: Scatchard analysis after the treatment for hypoglycemia (2011).

After recovery from the DKA, he was treated with Novolin R and N, but his blood glucose control was brittle so he was transferred to our hospital in July.

At the time of admission to Ehime University Hospital, an itchy wheal-flare was observed at the insulin injection sites (Fig. 3a). Laboratory findings were as follows (Table 1): fasting blood glucose 238 mg/dL, HbA1c 12%, postprandial 2 hours serum C-peptide 0.2 ng/mL. Islet-related autoantibodies (ICA), GAD Ab, and IA-2 Ab were negative. He was positive for an insulin-specific IgE antibody (3.16 UA/mL), and a high titer of insulin antibody (89%) was detected. Because of the insulin allergy, his insulin was changed to insulin aspart (NovoRapid; Novo Nordisk) of CSII. However, 4 days later, a 2×2-cm itchy wheal-flare and a nodule appeared at the insulin injection sites. We then switched from NovoRapid to Humalin R (Eli Lilly) by continuous intravenous insulin infusion therapy.

The next day, an itchy wheal-flare, repeatedly appeared on the abdomen, thighs and upper arms. Desensitization with NovoRapid was performed in August, and NovoRapid by CSII and anti-allergic agents were started. The intensity of the itchy wheal-flare then decreased, but his blood glucose control was still unstable, and nocturnal hypoglycemia frequently occurred despite his insulin dose being adjusted and the intake of extra food initiated (Fig. 3b).

Since a high titer of insulin antibody was detected (Table 1), we performed a Scatchard analysis of the IAb, as described for Case 1 (Fig. 4a). It showed an extremely lower affinity constant (K1) and an extremely higher binding capacity (B1) at a high-affinity, low-capacity population with the same characteristics as in Case 1 as well as with IAS (5). As described in the Case Report section, we reconducted the Scatchard analysis using human insulin from serum stocked at the onset. The K1 and B1 values were nearly the same when either human or pig insulin was used: K1, 0.00188 vs. 0.0194×10⁻⁸ M⁻¹; B1, 140 vs. 261×10⁻⁸ M, using human vs. pig insulin (15), respectively. These data suggest that IAb might cause nocturnal hypoglycemia and daytime hyperglycemia, as described in Case 1.

In September, we performed a pancreatic biopsy, and he was diagnosed with insulin-triggered T1DM with typical autoimmune insulitis (15, 16). On October, CSII was changed to MDI using NovoRapid before each meal without basal insulin at bedtime. These preparations improved the nocturnal hypoglycemia and the daytime hyperglycemia as well, as shown in Fig. 3c. Furthermore, the insulin allergy gradually improved and finally disappeared without any anti-allergic drugs and he was discharged in November.

In 2011, he was readmitted to Ehime University Hospital because of morning hyperglycemia. The clinical characteristics and laboratory findings at readmission were as follows: BMI, 21.8; HbA1c, 8.8%; and serum C-peptide <0.03 ng/mL. GAD Ab and IA-2 Ab were still negative (Table 1). The insulin preparation was the same insulin analog as at his first admission. When we changed the doses and timing of insulin injections using the same insulin analog, his blood glucose control improved (Fig. 3d). The high titer of IAb decreased slightly from 89% to 78.8%, but the K1 and B1 values on Scatchard analyses markedly improved (Fig. 4b). These results again suggested that the changes in the Scatchard analysis findings were consistent with the changes in the hypoglycemic attacks, as described above. The IAb titer decreased to 60% in 2014 but increased to 88.7% in 2018. In 2014, we added insulin glargine (Lantus; Sanofi) in the evening as basal insulin to prevent early-morning hyperglycemia. These practices further improved his blood glucose profile.

Neither of these two patients had received sulfhydryl-containing compounds or had IAS-susceptible HLA class II, *DRB1*04:06* (3), suggesting that the present cases were distinct from IAS.

The two patients were informed of the purpose of the study, and their written informed consent was obtained. The study was approved by the ethics committee of Ehime University Hospital and the Ehime University Graduate School of Medicine and was carried out in accordance with the Declaration of Helsinki.

Discussion

IAb production due to the administration of exogenous insulin reportedly differs from the route of insulin administra-

tion. Both implantable insulin pumps and inhaled insulin have been associated with increases in IAb levels (2). While CSII subjects develop IAb more frequently than MDI, IAb does not impair the glycemic control in either group (17). Cases of recurrent hypoglycemia due to IAb production with MDI or conventional insulin therapy have been sporadically reported in Japan, as described above (8-12, 18-44). We therefore conducted a literature search for relevant studies regarding recurrent hypoglycemia due to IAb production with CSII in the ICHUSHI (a domestic medical literature database service provided by the NPO Japan Medical Abstracts Society), the PubMed database and the Journal of the Japan Diabetes Society. We found only one abstract that described a 69-year-old woman with T1DM who developed recurrent hypoglycemia due to CSII 8 months later (45). Therefore, Case 1 is the first case report, not in abstract form, of a subject presenting with recurrent hypoglycemia due to IAb production using CSII. The low incidence rate of these cases may be related to the low usage of CSII in T1DM patients compared with that of MDI or conventional insulin therapy in either T1DM or T2DM patients. Furthermore, this case was not one of treatment failure, as he was able to manage both CSII as well as carbohydrate counting by himself, and his HbA1c levels remained acceptable until the onset of the hypoglycemic episodes. Case 2 was one of three or six cases of insulin-triggered T1DM that have been previously reported (15, 16), but only the present case simultaneously developed recurrent hypoglycemia as well as insulin-triggered T1DM, the reasons for which are not currently known.

Scatchard analyses are widely used to determine the variance of affinities and the binding capacity of IAbs. Such analyses usually show a curvilinear relationship with two classes of binding sites: a high-affinity (K1), low-capacity population (B1) and a low-affinity (K2), high-capacity population (B2) (Fig. 2, 4). The characteristics of the IAA of IAS reportedly show an extremely lower affinity constant (K 1) and a higher binding capacity (B1) at a high-affinity, low-capacity population compared with control insulin-treated diabetic patients (5) (Table 3). The present two cases also showed an extremely lower affinity constant (K1) and a higher binding capacity (B1) before treatment (at onset), similar to that of IAS (Fig. 2a, 4a). Depending on the improvement in recurrent hypoglycemia, in both cases, the K1 value became slightly or greatly increased and the B1 value greatly decreased compared with the corresponding values before treatment (Fig. 2b, 4b). The changes in the Scatchard analysis results after treatment were consistent with the changes in hypoglycemic attacks compared with the changes in insulin binding. These characteristics were consistent with reports of hypoglycemic cases due to the IAb production in T1DM and T2DM patients, as reported previously (8, 12, 24, 25, 29-31, 33, 34, 36, 38, 40, 42-44).

Similar changes in Scatchard analysis findings concomitant with an improvement in hypoglycemic attacks have been reported twice in a case of IAS with granuloma due to the use of gold thioglucose by Eguchi et al. (14). When hypoglycemia was severe, the total immunoreactive insulin (IRI) levels was elevated, and a Scatchard analysis showed a relatively low affinity constant (K1) and very high binding capacity (B1). When the attacks were relieved by the treatment, the total IRI level was decreased, and the K1 and B1 value showed a higher affinity constant and a lower binding capacity. This indicates that the K1/B1 population of IAb may easily release human insulin into the serum, leading to the development of hypoglycemia. The authors further suggested that B cell clones may have been changed during the clinical course, possibly due to somatic hypermutation. This phenomenon may also have occurred in the clinical course of the present two cases.

HLA class II-DR4 or HLA class II-DR7 has been reported to be associated with IAb formation (2) and HLA class II-DR8 with the hypoglycemic cases (23). In the present study T1DM susceptible DRB1-DQB1 haplotypes, DRB1*04:05-DQB1*04:01 and/or DRB1*09:01-DQB1*03: 03 (46, 47) were observed in both Case 1 and Case 2 (Table 1) (15, 16). We previously reported on the existence of a relationship between insulin-triggered T1DM and the insulin gene VNTR class I genotype (*IDDM2*) (15, 16) as well as the *BACH2* gene SNP (48), both of which are risk variants for T1DM. We identified these in Case 1 in addition to Case 2 (48), as shown in Table 1. However, further studies will be necessary to clarify the genetic background responsible for these hypoglycemic cases.

In most recurrent hypoglycemic cases, insulin cessation or switching the insulin formulation and/or the use of oral hypoglycemic agents (OHA) has been reported to be a successful form of treatment (10, 18, 20-22, 24-44). Glucocorticoid administration has also been used for the treatment of either T1DM or T2DM cases (8, 9, 12, 19, 23, 31, 42). As described in these case reports, we decided to switch the insulin formulation and change from CSII to MDI in Case 1 and only changed from CSII to MDI in Case 2 without glucocorticoid administration. These results suggest that, in both cases, stopping basal infusion at night for CSII might be effective in preventing nocturnal hypoglycemia. However, in Case 1, we were unable to exclude the effect of switching the insulin formulation, as the term of observation was only three days. Thus far, there has been no definite protocol for the treatment of such hypoglycemic cases. Regarding other treatments, cyclophosphamide (19), double-filtration plasmapheresis (19, 23, 33, 42), a GLP-1 analogue (26, 30, 32, 37, 40-43) or a SGLT-2 inhibitor (38, 39, 41) have been reported. An interesting case report was recently published reporting that the administration of sodium bicarbonate improved metabolic acidosis as well as morning hypoglycemia (44).

In conclusion, the clinical characteristics and Scatchard analyses data for the recurrent hypoglycemic cases due to a high titer of IAb are essentially the same as those for IAS, except for insulin administration. Since these cases appear to occur sporadically, there is not much chance that they will

be encountered, especially in cases of CSII treatment. Therefore, if insulin-treated patients show recurrent hypoglycemia without any cause other than a high titer of IAb, it would be advisable to diagnose them correctly using Scatchard analyses as described above and then treat them appropriately.

References

1. Klingensmith GJ. Insulin antibodies - are they still with us? Do they matter? Pediatr Diabetes **12**: 75-77, 2011.
2. Fineberg SE, Kawabata TT, Finco-Kent D, Fountaine RJ, Finch GL, Krasner AS. Immunological responses to exogenous insulin. Endocr Rev **28**: 625-652, 2007.
3. Uchigata Y, Eguchi Y, Takayama-Hasumi S, Omori Y. Insulin autoimmune syndrome (Hirata disease): clinical features and epidemiology in Japan. Diabetes Res Clin Pract **22**: 89-94, 1994.
4. Hirata Y, Ishizu H, Ouchi N, et al. Insulin autoimmunity in a case with spontaneous hypoglycemia (in Japanese). J Jpn Diab Soc **13**: 312-320, 1970.
5. Eguchi Y. Scatchard analysis of insulin autoantibodies in the insulin autoimmune syndrome (in Japanese). J Tokyo Wom Med Coll **59**: 1296-1305, 1989.
6. Uchigata Y, Kuwata S, Tokunaga K, et al. Strong association of insulin autoimmune syndrome with HLA-DR4. Lancet **339**: 393-394, 1992.
7. Harwood R. Insulin-binding antibodies and "spontaneous" hypoglycemia. N Engl J Med **262**: 978-979, 1960.
8. Matsuyoshi A, Shimoda S, Tsuruzoe K, et al. A case of slowly progressive type 1 diabetes with unstable glycemic control caused by unusual insulin antibody and successfully treated with steroid therapy. Diabetes Res Clin Pract **72**: 238-243, 2006.
9. Yamashita T, Okada S, Kohmoto J, et al. Chomei na koukettou to souchou teikettou wo kurikaeshita kou insurin koutai yousei tounyoubyou no ichi rei [A case of diabetes with repeated hyperglycemia and morning hypoglycemia caused by insulin antibody] (in Japanese). J Jpn Diab Soc **45**: 319-323, 2002.
10. Sakamoto N, Sakamoto Y, Maruyama M, Tsuruoka A, Uchigata Y, Tajima N. A type 1 diabetic patient with prolonged fasting hypoglycemia and postprandial hyperglycemia due to insulin autoimmune syndrome like insulin antibodies. Evaluation by Scatchard analysis (in Japanese). J Jpn Diab Soc **45**: 471-476, 2002.
11. Koriyama N, Hashiguchi H, Aso K, et al. A type 1 diabetes case in the aged onset in basedow's disease course with continuous elevation of insulin autoimmune syndrome like insulin antibodies (in Japanese). J Jpn Diab Soc **46**: 801-806, 2003.
12. Honda M, Kawashima Y, Kawamura H, et al. Acute liver dysfunction complicated with uncontrollable glycemia due to insulin antibody: successful treatment with glucocorticoid and lispro insulin. Intern Med **45**: 1225-1229, 2006.
13. Scatchard G. The attractions of proteins for small molecules and ions. Ann N Y Acad Sci **51**: 660-672, 1949.
14. Eguchi Y, Uchigata Y, Yao K, Yokoyama H, Hirata Y, Omori Y. Longitudinal changes of serum insulin concentration and insulin antibody features in persistent insulin autoimmune syndrome (Hirata's disease). Autoimmunity **19**: 279-284, 1994.
15. Nakamura M, Nishida W, Yamada Y, et al. Insulin administration may trigger pancreatic beta-cell destruction in patients with type 2 diabetes. Diabetes Res Clin Pract **79**: 220-229, 2008.
16. Nishida W, Nagata M, Imagawa A, et al. Insulin administration may trigger type 1 diabetes in Japanese type 2 diabetes patients with type 1 diabetes high-risk HLA class II and the insulin gene VNTR genotype. J Clin Endocrinol Metab **99**: E1793-E1797, 2014.
17. Sahin SB, Cetinkalp S, Ozgen AG, Saygili F, Yilmaz C. The importance of anti-insulin antibody in patients with type 1 diabetes mellitus treated with continuous subcutaneous insulin infusion or multiple daily insulin injections therapy. Acta Diabetol **47**: 325-330, 2010.
18. Yamada M, Fujimoto K, Yoshihara R, et al. A case report of fasting hypoglycemia and daytime hyperglycemia due to insulin antibodies using continuous glucose monitoring (CGM) in type 1 diabetes (in Japanese). J Jpn Diab Soc **51**: 791-796, 2008.
19. Ishizuka T, Ogawa S, Mori T, et al. Characteristics of the antibodies of two patients who developed daytime hyperglycemia and morning hypoglycemia because of insulin antibodies. Diabetes Res Clin Pract **84**: e21-e23, 2009.
20. Kabeya Y, Mokubo A, Tomita M, et al. A patient with mitochondrial diabetes with acute exacerbation of glycemic control due to insulin autoimmune syndrome-like insulin antibodies (in Japanese). J Jpn Diab Soc **52**: 39-43, 2009.
21. Tanaka H, Uchigata Y, Fujioka M, et al. A case of type 2 diabetes mellitus whose blood glucose became uncontrolled with the development of insulin antibody after short acting insulin-analogue treatment (in Japanese). J Jpn Diab Soc **52**: 561-567, 2009.
22. Kunugi T, Jimba S, Yanagisawa K, et al. A case of elderly slowly progressive type 1 diabetes with frequent hypoglycemia due to insulin antibody and changed to oral hypoglycemic agents (in Japanese). Diabetes J **38**: 103-108, 2010.
23. Sekiya K, Kurihara S, Yagi S, et al. Two cases of type 2 diabetes with unstable glycemic control caused by insulin antibodies against therapeutic pre-mixed insulin analog: case presentation and speculation of the etiology (in Japanese). J Jpn Diab Soc **54**: 361-368, 2011.
24. Itoh A, Saisho Y, Mitsuishi M, et al. Insulin glulisine may ameliorate nocturnal hypoglycemia related to insulin antibody - a case report. Diabetes Res Clin Pract **94**: e53-e54, 2011.
25. Fukushima T, Harada N, Sasaki M, et al. Improvement of recurrent unstable glycemic control using continuous glucose monitoring (CGM) in a patient with diabetes complicated by insulin antibodies (in Japanese). J Jpn Diab Soc **55**: 966-972, 2012.
26. Sato A, Ohama T, Tanaka S, Ishizuka T, Katsumori K. A case of insulin antibody-positive type 2 diabetes mellitus effectively treated with a GLP-1 receptor agonist and assessed in continuous glucose monitoring (CGM) (in Japanese). J Jpn Diab Soc **55**: 398-403, 2012.
27. Nagao M, Nakajima Y, Sato Y, et al. Evaluation of glycemic variation with continuous glucose monitoring in unstable diabetes caused by anti-insulin antibodies. J Jpn Diab Soc **55**: 874-879, 2012 (in Japanese).
28. Iizuka K, Tomita R, Horikawa Y, Takeda J. A case of glycemic instability and insulin allergy due to anti-insulin antibodies in a patient with type 2 diabetes. Diabetol Int **3**: 233-238, 2012.
29. Takebe R, Odake Y, Muramae N, Okada Y, Nakamura T. A case of type 2 diabetes mellitus with frequent hypoglycemia due to insulin antibodies after switching insulin product (in Japanese). Nihon Naika Gakkai Zasshi (J Jpn Soc Intern Med) **102**: 2976-2979, 2013.
30. Okada Y, Kobayashi H, Shiraki S, et al. A case of diabetes mellitus with early morning hypoglycemia induced by insulin antibodies successfully treated with a combination of liraglutide and insulin analogue (in Japanese). J Jpn Diab Soc **56**: 570-577, 2013.
31. Ando A, Yamane T, Suzuki Y. Insulin antibody properties and its

change induced by insulin detemir in type 2 diabetic patients with recurrent hypoglycemic episodes: a report of two cases (in Japanese). Nihon Naika Gakkai Zasshi (J Jpn Soc Intern Med) **102**: 437-439, 2013.

32. Tamura Y, Kimbara Y, Funatsuki S, et al. A case of insulin antibody-induced glucose instability in an elderly woman with type 2 diabetes on hemodialysis, successfully ameliorated with liraglutide. Diabetol Int **4**: 71-75, 2013.

33. Nishikawa M, Teranishi T, Morisawa T, et al. Successful long-lasting treatment with double-filtration plasmapheresis in a case of type 2 diabetes with anti-insulin antibodies (in Japanese). Nihon Toseki Igakkai Zasshi (J Jpn Soc Dial Ther) **47**: 159-165, 2014.

34. Osugi T, Toyoda K, Okamura E, Hamasaki A, Inagaki N. Insulin allergy and unstable glycemic control due to insulin antibodies in a patient with type 2 diabetes treated with insulin analogs (in Japanese). J Jpn Diab Soc **57**: 438-445, 2014.

35. Saiga M, Nishino M, Tanaka H, et al. Jizoku kettou monita de hyouka shi α-GI to sokkou gata insurin bunpitsu sokushin yaku ga yuukou de atta insurin koutai yousei 2 gata tounyoubyou no 1 rei [A case of type 2 diabetes with insulin antibody effectively treated with α-GI and glinide assessed by continuous glucose monitoring] (in Japanese). Diabetes Front **25**: 491-495, 2014.

36. Hirata Y, Hirota Y, Odake Y, et al. A case of the insulin antibody-positive type 2 diabetes patient with assessing the antibody characteristics by scatchard analysis during 4 years (in Japanese). J Jpn Diab Soc **58**: 842-849, 2015.

37. Amari Y, Ohyama Y, Yurugi T, et al. A case of insulin antibody-positive type 2 diabetes mellitus in a hemodialysis patient effectively treated with a GLP-1 receptor agonist (Liraglutide) (in Japanese). J Osaka Soc Dial Ther **33**: 47-50, 2015.

38. Hayashi A, Takano K, Kawai S, Shichiri M. SGLT2 inhibitors provide an effective therapeutic option for diabetes complicated with insulin antibodies. Endocr J **63**: 187-191, 2016.

39. Koshibu M, Satoh H, Satoh J, et al. Insurin koutai yousei huantei-gata tounyoubyou ni SGLT2 sogaiyaku wo motiita itirei [A case of unstable diabetes with insulin antibody treated with SGLT2 inhibitor] (in Japanese). Tokyo Naika Ikai Kaishi **33**: 171-175, 2017.

40. Ueno S, Watanabe K, Kubota S, et al. Serial changes in characteristics of anti-insulin auto antibody in a patient with diabetes mellitus (in Japanese). Diabetes J **46**: 159-164, 2018.

41. Tokuyama Y, Yanagisawa Y. A case of type 2 diabetes in which the combination therapy of dulaglutide and tofogliflozin was significantly effective for blood glucose fluctuation due to insulin antibody (in Japanese). J Jpn Diab Soc **61**: 307-314, 2018.

42. Taya N, Kato K, Oida T, Mitsui E, Taki H. A case of diabetes mellitus with fasting hyperglycemia due to changes in the characteristics of insulin antibodies after the administration of an insulin analogue (Glargine) (in Japanese). J Jpn Diab Soc **62**: 170-177, 2019.

43. Kaneko K, Satake C, Izumi T, et al. Enhancement of postprandial endogenous insulin secretion rather than exogenous insulin injection ameliorated insulin antibody-induced unstable diabetes: a case report. BMC Endocr Disord **19**: 1-7, 2019.

44. Kobayashi S, Amano H, Kawaguchi Y, Yokoo T. A novel treatment of hyperinsulinemic hypoglycemia induced by insulin antibodies with alkali administration: a case report. J Med Case Rep **13**: 79, 2019.

45. Urai S, Hirota Y, Nishimoto Y, et al. CSII dounyuu go insulin koutai ni yoru kettou control akka wo mitometa 1 gata tounyou byou no 1 rei [A case of type 1 diabetes with deterioration in glycemic control due to insulin antibody after induction of CSII] (in Japanese). J Jpn Diab Soc **58**: 493, 2015 (Abstract).

46. Takeda H, Kawasaki E, Shimizu I, et al. Clinical, autoimmune, and genetic characteristics of adult-onset diabetic patients with GAD autoantibodies in Japan (Ehime Study). Diabetes Care **25**: 995-1001, 2002.

47. Sugihara S, Ogata T, Kawamura T, et al. HLA-class II and class I genotypes among Japanese children with Type 1A diabetes and their families. Pediatr Diabetes **13**: 33-44, 2012.

48. Onuma H, Kawamura R, Tabara Y, et al. Variants in the BACH2 and CLEC16A gene might be associated with susceptibility to insulin-triggered type 1 diabetes. J Diabetes Investig **10**: 1447-1453, 2019.