

ORIGINAL

Trajectories of anti-islet autoantibodies before development of type 1 diabetes in interferon-treated hepatitis C patients. Case reports and a literature review

Kan Nakamura¹⁾, Eiji Kawasaki¹⁾, Norio Abiru²⁾, Ozora Jo²⁾, Keiko Fukushima²⁾, Tsuyoshi Satoh²⁾, Genpei Kuriya²⁾, Masakazu Kobayashi²⁾, Hironaga Kuwahara²⁾, Hironori Yamasaki³⁾, Tatsuya Ide⁴⁾ and Katsumi Eguchi^{1), 2)}

¹⁾Department of Metabolism/Diabetes and Clinical Nutrition, Nagasaki University Hospital, Nagasaki, Japan

²⁾First Department of Internal Medicine, Graduate School of Biomedical Science, Nagasaki University, Nagasaki, Japan

³⁾Center for Health and Community Medicine, Nagasaki University, Nagasaki, Japan

⁴⁾Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Fukuoka, Japan

Abstract. Interferon-alpha (IFN- α) is widely used in the treatment of viral hepatitis, however, it is known that IFN- α therapy may induce type 1 diabetes. We report here on two cases of chronic viral hepatitis C who developed autoimmune type 1 diabetes during Peg-IFN- α plus ribavirin (RBV) therapy. *Case 1:* a 48-year-old male with chronic hepatitis C with chronic thyroiditis. The patient's plasma glucose level was normal and anti-islet autoantibody tests were negative before Peg-IFN- α +RBV therapy. The emergence of glutamic acid decarboxylase 65 autoantibody (GAD65Ab) was observed after five months of treatment. Autoantibodies to insulin and insulinoma-associated antigen-2 (IA-2) also became positive. Eleven months later, thirst and polydipsia occurred with increased fasting plasma glucose level and the patient was diagnosed with type 1A diabetes. Zinc transporter-8 autoantibody (ZnT8Ab) was not detectable at any point. The patient has type 1 diabetes-susceptible HLA-DRB1-DQB1 haplotypes *0405-*0401 and *0901-*0303. *Case 2:* a 65-year-old male with chronic hepatitis C with type 2 diabetes on insulin treatment. GAD65Ab and IA-2Ab were negative before Peg-IFN- α +RBV therapy, however, nine months later, a single appearance of GAD65Ab was observed. After twelve months, his plasma glucose control worsened rapidly, and he was diagnosed with type 1A diabetes. IA-2Ab and ZnT8Ab were negative throughout the clinical course. His HLA-DRB1-DQB1 haplotypes were *0410-*0402 and *1407-*0503. Both cases showed a unique GAD65Ab epitope (amino acids 360-442). These clinical courses suggest that IFN- α therapy provoked acute islet autoimmunity and onset of type 1 diabetes. Therefore, during IFN- α therapy, patients should be closely monitored for the occurrence of type 1 diabetes.

Key words: Type 1 diabetes, Interferon, Glutamic acid decarboxylase 65 autoantibody, HLA

INTERFERON-ALPHA (IFN- α) is widely used in the treatment of viral hepatitis, renal cell carcinoma, chronic myelogenous leukemia, and multiple myeloma for the induction of antiviral proteins and activation of natural killer cells [1]. It is also known that IFN- α therapy may trigger the development of type 1 diabetes, and various patients who developed type 1 diabetes during IFN- α therapy have been reported

[2-12]. In this paper, we report on the time course of anti-islet autoantibodies in two cases with type 1 diabetes that developed after Peg-IFN- α +ribavirin (RBV) therapy for chronic hepatitis C.

Case 1

A 48-year-old Japanese male with chronic hepatic

Received Jul. 8, 2010; Accepted Aug. 6, 2010 as K10E-207

Released online in J-STAGE as advance publication Aug. 26, 2010

Correspondence to: Eiji Kawasaki, M.D., Ph.D., Department of Metabolism/Diabetes and Clinical Nutrition, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.

E-mail: eijikawa@nagasaki-u.ac.jp

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Abbreviations: Ab, autoantibody; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DKA, diabetic ketoacidosis; FPG, fasting plasma glucose; GAD, glutamic acid decarboxylase; HbA_{1c}, hemoglobin A1c; IA-2, insulinoma-associated antigen-2; IAA, insulin autoantibody; IFN, interferon; RBV, ribavirin; ZnT8, zinc transporter-8.

tis C and chronic thyroiditis had received IFN therapy twice in the past. He had no family history of diabetes mellitus. By January 2007, he had undergone IFN therapy a total of three times (Peg-IFN- α 2b: Intron A[®] +RBV: Rebetol[®]). Before the IFN- α therapy, his hepatitis C virus (HCV) serotype was group 1 and his viral titer was 510 KIU/mL. His plasma glucose level was normal and anti-islet autoantibody tests were negative before treatment. Two months after beginning the treatment, his HCV RNA became negative, and ten months after beginning the treatment, he noticed thirst, polydipsia and polyuria, and was admitted to our hospital. His laboratory data on admission were: fasting plasma glucose (FPG), 154 mg/dL; hemoglobin A1c (HbA_{1c}), 7.6%; aspartate aminotransferase (AST), 27 IU/L; alanine aminotransferase (ALT), 27 IU/L; HCV-RNA, negative; anti-thyroglobulin antibodies, positive ($\times 25600$); and anti-thyroid microsomal antibodies, positive ($\times 6400$). Fasting serum C-peptide level (3.39 ng/mL) and urinary C-peptide excretion (77 μ g/day), were retained. With respect to anti-islet autoantibodies, three of the four analyzed autoantibodies were positive: glutamic acid decarboxylase (GAD)65Ab, 1227 U/mL (normal value <1.4 U/mL); insulinoma-associated antigen-2 (IA-2)Ab, 10.6 U/mL (<0.4 U/mL); and insulin autoantibody (IAA), 1026.1 nU/mL (<125 nU/mL). However, zinc transporter-8 (ZnT8)Ab were negative. The patient's human leukocyte antigen (HLA) haplotypes were DRB1*0405-DQB1*0401 and DRB1*0901-DQB1*0303. Type 1 diabetes was diagnosed and antiviral treatment was withdrawn. Insulin therapy was then initiated.

To determine whether the appearance of anti-islet autoantibodies preceded Peg-IFN- α +RBV therapy, earlier samples were screened for GAD65Ab, IAA, IA-2Ab and ZnT8Ab. Retrospective serology revealed that GAD65Ab was positive after five months, IAA was positive after six months and IA-2Ab was positive after nine months from the initiation of IFN- α therapy (Table 1). In spite of terminating the IFN- α treatment, the patient's C-peptide response to 1 mg i.v. glucagon progressively decreased (Table 2).

Case 2

A 65-year-old Japanese male with chronic hepatitis C and type 2 diabetes had been on insulin treatment for nine years. He had received IFN therapy twice in the past. He had no family history of diabe-

tes mellitus. By January 2007, he had received Peg-IFN- α +RBV therapy a total of three times (Peg-IFN- α 2b: Intron A[®] +RBV: Rebetol[®]). Eight months after beginning IFN- α therapy, his HCV RNA became negative. Twelve months after beginning treatment, his plasma glucose control worsened rapidly, and he was admitted to our hospital. His laboratory data on admission were: FPG, 91 mg/dL; HbA_{1c}, 8.4%; AST, 31 IU/L; ALT, 28 IU/L; HCV-RNA, negative; thyroid peroxidase antibodies, 113 U/mL (<0.3 U/mL); and thyroid stimulating hormone receptor antibodies, 6.7 IU/mL (1.0 IU/mL). His fasting serum C-peptide level (0.52 ng/mL) and urinary C-peptide excretion (13.2 μ g/day) were decreased. GAD65Ab (3520 U/mL) was positive, but IA-2Ab and ZnT8Ab were negative. IAA was not measured because of his insulin treatment before the onset of type 1 diabetes. The patient's HLA haplotypes were DRB1*0410-DQB1*0402 and DRB1*1407-DQB1*0503. Type 1 diabetes was diagnosed and antiviral treatment was withdrawn.

Anti-islet autoantibodies were analyzed using stored sera, revealing that GAD65Ab was positive after nine months from the initiation of IFN- α therapy (Table 1). The patient's C-peptide response to 1 mg i.v. glucagon had been exhausted by the time of the diagnosis of type 1 diabetes (Table 2).

Both patients' GAD65Ab epitope recognition was analyzed using the GAD65/GAD67 chimeric proteins as previously described [13]. Both cases reacted with a unique epitope between amino acids 360-442 of GAD65. Furthermore, the GAD65Ab epitope spread to the C-terminal region (amino acids 443-585) at the onset of type 1 diabetes in Case 2.

Discussion

To the best of our knowledge, this is the first report that describes the time course of anti-islet autoantibodies before the onset of type 1 diabetes induced by IFN therapy in Japanese where the incidence of type 1 diabetes is one of the lowest ethnic groups in the world. In both of our two cases, anti-islet autoantibodies emerged rapidly after the initiation of IFN- α treatment for chronic hepatitis C. After several months from the development of anti-islet autoimmunity, the patients' plasma glucose level was elevated and type 1 diabetes was diagnosed.

Table 3 summarizes the anti-islet autoantibody profile before and after IFN therapy as well as class II

Table 1 Time course of anti-islet autoantibodies in two cases.

	Time (months)	GAD65Ab (U/mL)	IA-2Ab (U/mL)	IAA (nU/mL)	ZnT8Ab (index)	Event
Case 1						
	0	negative	negative	negative	negative	HCV-RNA+
	1	negative	negative	negative	negative	HCV-RNA+
	2	negative	negative	negative	negative	HCV-RNA-
	3	negative	negative	negative	negative	HCV-RNA-
	4	negative	negative	negative	negative	HCV-RNA-
	5	8.5	negative	negative	negative	HCV-RNA-
	7	436	negative	482	negative	HCV-RNA-
	8	942	negative	668.4	negative	HCV-RNA-
	9	1230	4.1	1113.7	negative	HCV-RNA-
	11	1220	8.8	1026.1	negative	T1D onset
	13	1277	10.6	N.D.	N.D.	
Case 2						
	0	negative	negative	N.D.	negative	HCV-RNA+
	1	negative	negative	N.D.	negative	HCV-RNA+
	2	negative	negative	N.D.	negative	HCV-RNA+
	3	negative	negative	N.D.	negative	HCV-RNA+
	4	negative	negative	N.D.	negative	HCV-RNA+
	5	negative	negative	N.D.	negative	HCV-RNA+
	6	negative	negative	N.D.	negative	HCV-RNA+
	9	72.1	negative	N.D.	negative	HCV-RNA+
	10	582	negative	N.D.	negative	HCV-RNA-
	12	3950	negative	N.D.	negative	T1D onset

N.D., not determined. Time = months after the initiation of IFN+RBV therapy. Ab, autoantibody; GAD, glutamic acid decarboxylase; IA-2, insulinoma-associated antigen-2; IAA, insulin autoantibody; ZnT8, zinc transporter-8

Table 2 C-peptide response to 1 mg i.v. glucagon.

	Time (min)	0	1	3	5	10	15
Case 1 (onset after 7 months)	CPR (ng/mL)	3.39	3.85	5.73	5.03	4.35	3.43
		1.52	2.17	3.28	3.42	2.77	2.72
Case 2 (onset)	CPR (ng/mL)	0.08	0.08	0.10	0.09	0.10	0.10

HLA in 17 patients with chronic viral hepatitis who developed type 1 diabetes and who have been reported in the literature and in the present two cases. Anti-islet autoantibody profiles at the onset of diabetes and HLA haplotypes are variable (Table 3). Nine of these 19 patients (47%) were positive for anti-islet autoantibodies before IFN treatment. Seven of the 19 patients (37%), including our two cases, seroconverted during treatment and all of them turned positive for GADAb. The remaining 3 cases (16%) were anti-islet autoanti-

body negative even after the onset of type 1 diabetes, although data for some autoantibodies such as ZnT8Ab were not available. These results suggest that GADAb may be a good predictive and diagnostic marker for IFN-induced type 1 diabetes, as has been reported in sporadic cases [2-12]. This needs to be verified in the future study using a large number of subjects.

The present Case 1 showed three of the four tested anti-islet autoantibodies, and susceptible HLA-DR-DQ haplotypes, and insulin secretion was retained at di-

Table 3 Anti-islet autoantibody profile before and after IFN therapy and class II HLA in patients with chronic viral hepatitis who developed type 1 diabetes.

Ref.	Age/ sex	IFN	Time (months)	Anti-islet autoantibodies		HLA
				Before IFN	At onset of type 1 diabetes	
2	61/M	α 2b	6	GAD (+), ICA (-), IAA (+), IA-2 (-)	GAD (+), ICA (+), IAA (+), IA-2 (-)	DRB1*0401/*1101, DQB1*0502/*0503
3	29/M	α 2b	5	GAD (+), ICA (+), IAA (-), IA-2 (-)	GAD (+), ICA (+), IAA (-), IA-2 (-)	DRB1*04/08, DQB1 57 N-Asp/Asp
4	57/M	α 2b	4	GAD (-), IAA (-)	GAD (-), ICA (-), IAA (-)	DRB1*0405/*1401, DQB1*0401/*0503
5	41/M	α 2b+RBV	3	GAD (-), IAA (-), IA-2 (-)	GAD (-), IAA (-), IA-2 (-)	DRB1*0101/*0401
5	36/F	α 2b+RBV	3	GAD (+)	GAD (+)	N.D.
6	29/M	α	8.5	GAD (-), ICA (-), IAA (-), IA-2 (-)	GAD (+), ICA (+), IAA (-), IA-2 (-)	DRB1*0301, DQB1*0201
7	37/M	α 2b+RBV	4	GAD (+), ICA (+)	GAD (+), ICA (+)	DR1/3
8	40/F	α 2b+RBV	6	GAD (+), ICA (-), IAA (-)	GAD (+)	DR4/7, DQ2/8
8	40/F	α 2b+RBV	2	GAD (+), ICA (-), IAA (-)	GAD (+)	N.D.
9	61/M	Peg- α 2b+RBV	3	GAD (+), ICA (+), IA-2 (-)	GAD (+), ICA (+), IA-2 (-)	DRB1*04/*14, DQB1*04/*0503
10	42/F	Peg- α 2b+RBV	2	GAD (-), ICA (-)	GAD (+), ICA (+)	DR1/4, DQ2/5
11	54/M	Peg- α +RBV	+1	GAD (-), ICA (-), IA-2 (-)	GAD (-), ICA (-), IA-2 (-)	DR3
11	46/M	Peg- α +RBV	3	GAD (+), ICA (-), IA-2 (-)	GAD (+), ICA (-), IA-2 (-)	N.D.
11	25/M	Peg- α +RBV	+1	GAD (-), ICA (-), IA-2 (-)	GAD (+), ICA (+), IA-2 (-)	DR3, DQ2
11	44/F	Peg- α +RBV	6	GAD (-), ICA (-), IA-2 (-)	GAD (+), ICA (-), IA-2 (-)	DR3/4, DQ2
11	46/M	Peg- α +RBV	4	GAD (+), ICA (+), IA-2 (-)	GAD (+), ICA (+), IA-2 (+)	N.D.
12	51/M	Peg- α 2b+RBV	6	GAD (-)	GAD (+), IA-2 (-)	N.D.
Case 1	48/M	Peg- α 2b+RBV	11	GAD (-), IAA (-), IA-2 (-), ZnT8 (-)	GAD (+), IAA (+), IA-2 (+), ZnT8 (-)	DRB1*0405/*0901, DQB1*0401/*0303
Case 2	65/M	Peg- α 2b+RBV	12	GAD (-), IA-2 (-), ZnT8 (-)	GAD (+), IA-2 (-), ZnT8 (-)	DRB1*0410/*1407, DQB1*0402/*0503

N.D., not determined; GAD, GADAb; ICA, islet cell antibody; IAA, insulin autoantibody; IA-2, IA-2Ab; ZnT8, ZnT8Ab.

Time = months after the initiation of IFN therapy; "+1" indicates one month after the end of IFN therapy.

agnosis. In contrast, Case 2 showed only GAD65Ab, has no susceptible HLA-DR-DQ haplotypes, and insulin secretion dried up upon the diagnosis of type 1 diabetes. Thus, it is clear that IFN- α is a common trigger of type 1 diabetes, but clinical courses vary greatly.

It has been reported that IFN- α is overexpressed in the pancreas of patients with type 1 diabetes [14]. Furthermore, in a study using transgenic mice, β cell-specific expression of IFN- α induced by using a monoclonal antibody protected mice from diabetes [15]. In addition, IFN- α is known to induce HLA class I antigen expression, and natural killer cell and T cell activities [16]. However, the underlying mechanisms of IFN-related type 1 diabetes have not yet been clarified. In contrast to the natural history of autoimmune type 1 diabetes, in which the appearance of anti-islet autoantibodies precedes the manifestation of insulin insufficiency by years, established humoral autoimmune markers were seen to have developed up to 3 to 6 months prior to diagnosis in the present cases. It is possible that such a rapid onset is due to acute β

cell destruction by IFN; this is supported by the fact that the prediction of type 1 diabetes is difficult in some cases. The nation-wide survey is being executed by Japan Diabetes Society to clarify the clinical and immunogenetic characteristics of IFN-related type 1 diabetes in Japan.

In the present cases, we recognized a unique GAD65Ab epitope. The GAD65Ab epitope located between amino acids 245-360 (E1) is thought to be the marker of acute β cell destruction [13, 17]. However, GAD65Ab E1 was negative and a novel epitope located between amino acids 360-442 was positive in our cases. These results suggest that the underlying mechanism of β cell destruction in patients with IFN-induced type 1 diabetes may be different from that in those with classical type 1A diabetes.

In Case 1, insulin secretion was remarkably decreased at seven months after the discontinuation of IFN- α therapy, suggesting that β cell destruction progressed in spite of the withdrawal of the therapy. In Case 2, insulin secretion had already dried up at the

time of the diagnosis of type 1 diabetes. However, since this patient had been treated with insulin for type 2 diabetes, he did not develop diabetic ketoacidosis (DKA).

In conclusion, the development of type 1 diabetes should be considered a side effect of IFN- α therapy. The onset of disease may be extremely abrupt; therefore, in order to protect patients from the risk of DKA

risk, patients receiving IFN- α therapy should be regularly monitored for the presence of anti-islet autoantibodies before and during IFN- α therapy.

Competing Interests

Nothing to declare.

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