

Fulminant type 1 diabetes associated with Isolated ACTH deficiency induced by anti-programmed cell death 1 antibody—insight into the pathogenesis of autoimmune endocrinopathy—

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Abstract. Therapeutic blocking antibodies against programmed death 1 (PD1) and cytotoxic T-lymphocyte antigen 4 (CTLA4) are applied for advanced cancer therapy, but induce a wide range of immune-related adverse events. In our recent case of a 52-year-old female doctor suffering from breast cancer having metastasized to the lung and liver, it was decided to use nivolumab to prevent the disease progressing after excisional surgeries and multiple chemotherapies. One month after completing the nivolumab course, fatigue, hypoglycemia and hypotension developed and isolated ACTH deficiency (IAD) was diagnosed. A further month later, under steroid supplementation, hyperglycemia emerged alongside thirst and polydipsia, prompting a diagnosis of fulminant type 1 diabetes (FT1D). Her susceptibility to type 1 diabetes was examined by HLA haplotype and CTLA4 gene polymorphism analyses. Polymorphisms CT60G>A and +49G>A in CTLA4 both generated a GG genotype. Our patient manifested one of the rarest combinations of autoimmune disease induced by nivolumab. Whereas the HLA haplotype was unsusceptible to autoimmune type 1 diabetes, polymorphisms of CTLA4, the antibody of which frequently causes hypophysitis, were susceptible to FT1D. Peripheral modulation of activated T cells, mainly by PD-1 antibodies, induced FT1D associated with IAD in patients with CTLA4 polymorphism. This case reveals hints of the T-cell etiology in T1D and evidence of CTLA4 involvement in IAD.

Key words: Fulminant type 1 diabetes, Isolated ACTH deficiency, Cytotoxic T-lymphocyte-associated protein 4, HLA, Nivolumab

THERAPEUTIC blocking antibodies against programmed death 1 (PD1) and cytotoxic T-lymphocyte antigen 4 (CTLA4), a pair of coinhibitory receptors expressed on activated T cells, are now applied as agents for routine clinical use in advanced cancer therapy. Moreover, combining the adoptive transfer of antitumor lymphocytes and anti-PD-1 antibody has reportedly helped attain the successful regression of metastatic breast cancer [1]. Recent studies have found, however, that immune checkpoint blockade with these antibodies induces inflammatory side effects affecting the endocrine organs or a condition resembling autoimmune endocrinopathy. Here, we report on the occurrence of the rarest combination of

autoimmune endocrinopathies, fulminant type-1 diabetes (FT1D) and isolated ACTH deficiency (IAD), induced by treatment with a total dose of 200 mg received over 6 months of nivolumab in a female patient suffering from breast cancer associated with metastasis to the lung and liver.

Case

A 52-year-old Japanese female doctor, who had been receiving nivolumab (20 mg/course, 0.36 mg/kg, biweekly) to treat breast cancer having metastasized to the lung and liver, was referred to our hospital due to general fatigue, hypoglycemia and hypotension. Eight years earlier, she underwent excisional surgery of the right breast to remove a cancer (T2N0M0, stage II, HER2 positive, hormone-receptor negative), followed by chemotherapy comprising 5-fluorouracil, epirubicin and cyclophosphamide with trastuzumab without steroid use.

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Table 1-a ACTH and CS responses to the 100 µg intravenous CRH load test

Time (min)	0	30	60	90	120
ACTH (pg/dL)	<2.0	<2.0	<2.0	<2.0	<2.0
Cortisol (µg/dL)	<1.0	<1.0	<1.0	<1.0	<1.0

CS, cortisol; ACTH, adreno-corticotrophic hormone; after 100 µg CRH (corticotropin-releasing hormone) given intravenously

Table 1-b ACTH, CS and glucose responses to the intravenous Insulin load test

Time (min)	0	30	60	90
ACTH (pg/dL)	3.5	3.8	3.3	3.4
Cortisol (µg/dL)	<1.0	<1.0	<1.0	<1.0
Glucose (mg/dL)	189	66	37	94

after 5 units humarin R[®] given intravenously

Table 1-c CS responses to the 25 µg rapid ACTH load test

Time (min)	0	30	60
Cortisol (µg/dL)	<1.0	1.6	1.8

after 25 µg ACTH given intravenously

Table 1-d GH responses to the 100 µg intravenous GHRH load test

Time (min)	0	15	30	45	60
GH (ng/mL)	0.22	24.2	34.7	27.5	18.6

GH, growth hormone; after 100 µg GHRH (growth hormone-releasing peptide) given intravenously

Table 1-e TSH responses to the 0.5 mg intravenous TRH load test

Time (min)	0	15	30	60	120
TSH (µU/mL)	2.5	13.8	16.0	11.5	5.9

TSH, thyroid-stimulating hormone; after 100 µg TRH (thyrotropin-releasing hormone) given intravenously

Table 1-f LH and FSH responses to the 100 µg intravenous LHRH load test

Time (min)	0	30	60	90
LH (mIU/mL)	24.1	77.4	92.8	ND
FSH (mIU/mL)	57.5	ND	87.5	94.9

LH, Luteinizing hormone; FSH, Follicle stimulating hormone; ND, not detected; after 100 µg LHRH (luteinizing hormone-releasing hormone) given intravenously

Soon after the treatment, she became menopausal and metastasis to the lung and liver was discovered three years after the surgery. Additional chemotherapy with capecitabine and vinorelbine without steroid use after partial lung resection under thoracoscopy caused the cancer to regress, but she decided to use nivolumab to prevent the disease progressing. The nivolumab was administered biweekly at a dosage of 0.36 mg/kg, 20 mg/course, resulting in a total dosage of 200 mg from November 2016 to April 2017. The patient had no complaints of subjective symptoms during these courses and the laboratory test values were within normal ranges at the end of treatment, including ACTH at 18.8 pg/mL and cortisol at 16.9 µg/dL. A month later, however, she started to complain of general fatigue, hypoglycemic symp-

toms and low blood pressure (86/52 mmHg). Laboratory tests revealed a low level of morning cortisol (2.8 µg/dL) with no corresponding increase in ACTH (1.5 pg/mL). We performed the load tests shown in Table 1 and the result of the rapid ACTH load test indicated secondary adrenal insufficiency. No responses in ACTH (<3.9 pg/dL) or cortisol (<1.0 µg/dL) were elicited during the CRH load test and insulin tolerance test at any point, nor any other abnormal hormonal responses from any other stimulant, which prompted a diagnosis of isolated ACTH deficiency. Other pituitary hormones were within normal ranges and an MRI revealed neither space-occupying lesions nor heterogeneous enhancement of the pituitary gland (data not shown). Hydrocortisone (15 mg/day) was started and continued.

Table 2 Laboratory Data on Admission for fulminant type 1 diabetes

glucose	402 mg/dL	[Antibody]	
HbA1c	7.8%	GAD	<5.0 U/mL
CPR	0.3 ng/mL	IA2	0.4 U/mL
[Urinary analysis]		insulin	<0.4 U/mL
pH	5.0	thyroid	
U-TP	(-)	Tg	13 U/mL
U-Glu	(3+)	TPO	10 U/mL
KET	(3+)	TR	<1.0 IU/mL
WBC	$7.5 \times 10^3/\mu\text{L}$	[Hormones]	
Seg	72.3%	ACTH	2.4 pg/mL
LYMPH	24.6%	Cortisol	<1.0 $\mu\text{g/dL}$
EOSIN	0.4%	PRL	19.5 ng/mL
RBC	$446 \times 10^6/\mu\text{L}$	LH	22.3 mIU/mL
Hb	13.0 g/dL	FSH	61.8 mIU/mL
Plt	$21.9 \times 10^4/\mu\text{L}$	AVP	5.0 pg/mL
[Arterial blood gas analysis]		PAC	112.0 pg/mL
pH	7.48	PRA	1.3 ng/mL/hr
cHCO ₃	17 mEq/L	TSH	1.28 $\mu\text{IU/mL}$
BE	-4.1	FT4	1.49 ng/mL
Na	131 mEq/L	[HLA-typing analysis]	
K	4.3 mEq/L	DRB1*	14:05, 14:06
Cl	92 mEq/L	DQB1*	03:01, 03:03
Anion Gap	22	[CTLA-4 polymorphism analyses]	
TP	7.7 g/dL	CT60G>A:	GG
Alb	4.4 g/dL	+49G>A:	GG
AST	25 IU/L		
ALT	11 IU/L		
BUN	18 mg/dL		
Cr	0.64 mg/dL		
AMY	64 IU		
Lipase	9 U/L		
CRP	0.69 mg/dL		

A month later, she visited our hospital with complaints of excessive thirst and polydipsia. Laboratory examinations (Table 2) indicated hyperglycemia (402 mg/dL) with ketosis associated with a rise in HbA1c to 7.8% as opposed to 5.9% a month earlier. Serum C-peptide was as low as 0.3 ng/mL and immunological studies were negative for anti-GAD and IA-2 antibodies. Her laboratory data were consistent with a diagnosis of fulminant type 1 diabetes mellitus. Throughout the course, her serum CPR level remained low, which is consistent with FT1DM. Her thyroid antibodies were also negative and

accompanied by a constant euthyroid status. Concerning partial diabetes insipidus, the patient's AVP level exceeded the normal range on admission and her urine volume was less than 2.5 L/day, at normal osmotic pressure (352 mOsm/kg H₂O) under normal serum osmolality (283 mOsm/kg H₂O) following supplementation of hydrocortisone and control of the blood glucose level. An MRI image of the pituitary gland showed iso-intensity in the T1-phase and these factors helped rule out masked partial diabetes insipidus.

Her susceptibility to type 1 diabetes was examined by

HLA haplotype and CTLA-4 gene polymorphism analyses, as shown in Table 2. Polymorphisms CT60G>A and +49G>A in CTLA-4 both generated a GG genotype.

Discussion

IAD is a rare autoimmune endocrinopathy [2-6], with reported incidence ranging from 3.8 to 7.3 per 100,000 persons over a decade-long 10-year period in Japan [7]. Regarding the association of IAD and T1D, three cases of IAD were reported in association with T1D in Japanese hospitals over the past 16 years, ranging from slowly progressive to fulminant, in a more recent paper [8].

Despite recent developments of antibodies for immune system checkpoints such as anti-PD1Abs and anti-CTLA4Abs showing a remarkable impact on advanced cancers, they also induce wide-ranging immune-related adverse events (irAEs). The irAEs can be useful in one respect, as they provide new insights into the pathogenesis of autoimmune endocrinopathy. Among irAEs in endocrine organs, the incidence of hypophysitis has appeared high in association with ipilimumab therapy, with as many as 15.6% of men and 3.6% of women receiving the agent respectively [9].

In contrast, no such association has been reported in patients receiving nivolumab ($\leq 1\%$) [10, 11]. The difference in antibody class, namely IgG1 for ipilimumab and IgG4 for nivolumab, is considered to dictate the divert frequency or manner of hypophysitis, by activating the complement pathway. Accordingly, anti-PD1 induced disorder mainly involves ACTH deficiency, as opposed to whole hypophysitis caused by anti-CTLA4 [12]. Animal studies and pathological analysis focused on the CTLA4 molecule have identified CTLA4 expression in the pituitary gland and tentatively characterized the molecule as a target for the anti-CTLA4 antibody [13, 14]. Another possibility reported is that autoimmunity is caused as a paraneoplastic syndrome, suggesting a different mechanism from immune checkpoint molecules [15]. The next step in elucidating the CTLA4 molecule will involve determining its role in the development of the isolated ACTH deficiency caused by nivolumab.

FT1D was reported in sequential treatment with anti-CTLA4Ab (ipilimumab), followed by anti-PD1Ab (pembrolizumab) [16]. A recent analysis showed that the incidence of T1D was as high as 0.2% based on the estimated number of patients treated with anti-PD1Ab (nivolumab) in Japan [17].

Our patient, manifesting the combination of IAD and FT1D induced by nivolumab, would be regarded as one of the rarest cases, a patient reportedly developing T1D and hypopituitarism by ipilimumab [18]. Facing this

case, we would review the characteristics of these autoimmune combinations in terms of molecular points to gain insights into the pathogenesis of the diseases.

The HLA haplotype, which we expected to be linked to the presentation of antigen to T cells, proved unsuitable to autoimmune T1D as well as immune checkpoint inhibitor induced T1D, for which the predominance of HLA-DR4 was suggested over spontaneous T1D [19]. The HLA haplotype alone does not appear sufficiently predictive of the induction of autoimmune diabetes by immune checkpoint cancer therapy [20]. PD-1, an inhibitory co-stimulatory molecule found on activated T cells, has been recently reported as playing a role in the pathogenesis of T1D. Failure of PD-1 upregulation upon T-cell receptor stimulation [21] and profound reductions in circulating CD4+PD-1+ and CD8+PD-1+ T cells were observed at the onset of the disease [22]. PD-1 is also reported to play a role in animal models of T1D [23]. Although the CTLA4 blockade induces the disease only in neonates, PD-1-PD-L1 interaction regulates the induction and progression of autoimmune diabetes in the NOD mouse at all ages, suggesting that PD-1 is involved both in inhibiting naïve T cells and the effector function of activated autoreactive T cells [23]. Regarding the role of CTLA4 in the development of autoimmune diabetes, polymorphisms of the allele reportedly exacerbate the risk of disease onset in pediatric and general population studies [24]. However, the risk allele of CTLA4 polymorphism for T1D or fulminant diabetes remains controversial. In the Japanese case, one report [25] suggested that the risk was exacerbated by CTLA4 polymorphism differently for FT1D compared to the classical T1D, only among the population with HLA-DR4(+).

Our case, however, presented HLA-DR4(−) and the risk allele reported in the pediatric and general population [24]; suggesting the existence of another mechanism of CTLA4 polymorphism involvement. Regarding the interaction of these molecules, although the working site on T cells differed, one report suggested regulating the soluble CTLA-4 concentration alongside the “G” code of CTLA-4 in patients with T1D [26].

While recent studies indicate that translational errors represent a major potential source of antigenic peptides for which central immune tolerance is lacking [27, 28], no homologous amino acid sequences emerged between proinsulin and pro-opiomelanocortin preproprotein. However, endocrine cells have multiple proteins in common through the hormone synthesis and secretory mechanism, which should have potential to be candidates that generate mismatched peptides leading to antigen presentation [1, 27, 28]. Further investigation of the association between FT1D and IAD will help fill gaps in our understanding of this mechanism.

We must now address the limitations of this discussion. This is simply a case report, which must be integrated and analyzed, in both animal models and a population study. A mechanistic insight into the pathogenesis of these autoimmune factors is also required.

In conclusion, peripheral modulation of activated T cells, mainly by PD-1 antibodies, induced FT1D with genetic susceptibility characterized by a CTLA4 polymorphism that might be associated with the rare disease IAD. An understanding of the exact mechanism behind the autoimmune endocrinopathy and the organ(s) poten-

tially involved should shed light on the interaction between immune tolerance and antigen expression on endocrine cells.

Disclosure Summary

Mitsubishi Tanabe Pharma, Daiichi Sankyo Company Limited, Ono Pharmaceutical Co., Ltd., Eli Lilly Japan KK, Boehringer Ingelheim, Merck Sharp & Dohme (MSD), Novartis Pharma, Novo Nordisk

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