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Brief report

Possible relevance of alpha lipoic acid contained in a health supplement in a case of insulin autoimmune syndrome

Noboru Furukawa, Nobuhiro Miyamura, Kenro Nishida, Hiroyuki Motoshima, Kayo Taketa, Eiichi Araki *

Department of Metabolic Medicine, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan

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Abstract

The insulin autoimmune syndrome is characterized as producing polyclonal or monoclonal anti-insulin autoantibodies in a patient with no previous history of exposure to exogenous insulin. The patient is 44-year-old Japanese woman and she had symptoms of hypoglycaemia without exposure to exogenous insulin. The patient was considered to have IAS because high titre of anti-insulin autoantibodies (96-98%: bound/total) were found in her serum. Her HLA DR β1 DNA sequences analysis revealed that she has the DRB1*0406 and DRB1*0901. Our patient have been taken alpha lipoic acid (ALA) before onset. SH group compounds are known to play an important role in the pathogenesis of IAS, and ALA contains SH. From these data, we propose the possibility of the correlation between pathogenesis of IAS and ALA, and it will be important to pay attention for ALA as a cause of hypoglycemia in such cases.

Keywords: Insulin autoimmune disease; Alpha lipoic acid

In September 2005, 44-year-old Japanese woman was admitted to our hospital. She had complained of recurrent attacks of weakness and malaise from mid August 2005. Her glucose values in these attacks had been in the range of 38–44 mg/dl, and immunoreactive insulin (IRI) levels were high with values of 330–538 μU/ml. Conventional imaging examination did not identify a pancreatic tumor. A high titre of anti-insulin autoantibodies (IAA) (96–98%: bound/total; normal range <10%) with low affinity ($k1 = 0.089 \times 10^8\ M^{-1}$) and high binding activity ($b1 = 10.0 \times 10^{-8}$ M) were found in her serum. The patient was considered to have insulin autoimmune syndrome (IAS), a rare autoimmune disorder producing polyclonal or monoclonal IAA in a patient with no previous history of exposure to exogenous insulin. Approximately, half of IAS patients have a history of medication prior to onset, and more than 90% of the agents are sulphydryl (SH) compounds such as methimazole, mercaptopropionyl glycine, or glutathione [1]. This patient had only recently, 2003–2004, taken norgesterel/Ethinylestradiol for oligomenorrhea, and had also taken a health and nutritional supplement containing alpha lipoic acid (ALA, 200 mg/day), Coenzyme Q10, and calnitin in June and from August 1st to 3rd, 2005. Among the drugs she had taken, only ALA belongs to the SH group. She stopped taking the supplement just before admission, and her symptoms disappeared without intervention.

* Corresponding author. Tel.: +81 96 373 5169; fax: +81 96 366 8397.
E-mail address: earaki@kaiju.medic.kumamoto-u.ac.jp (E. Araki).

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We reported a patient with IAS, who had been taking ALA for several weeks just before IAS developed. SH group compounds are known to play an important role in the pathogenesis of IAS, and several drugs are reported as being responsible. However, there has been no report of an IAS case induced by ALA. Because this supplement is currently used worldwide, it could be important to note that ALA might cause IAS. ALA is a natural compound, and with its reduced dithiol form, dihydrolipoic acid (DHLA), is a powerful antioxidant that contain SHs. The effects of ALA for improving diabetic peripheral and cardiac autonomic neuropathy, insulin sensitivity and cardiovascular disease have been reported [2,3]. Nevertheless, that our patient took ALA in June and from 1 to 3 August 2005, and that the hypoglycemic symptoms began from 15 August 2005, and that remission of the disease occurred shortly after withdrawal of the agent leads us to believe that ALA ingestion is the responsible factor in the present case.

Another factor, HLA, also needs to be considered. There are known to be interracial differences in HLA distribution. IAS occurs mainly in East Asia, especially in Japan, and one of the features is that the most patients possess DRB1*0406 (one of the HLA DRB1 molecules comprising the HLA-DR4 phenotype), which is believed to be crucial for the development of IAS. Matsushita et al. proposed a model of the pathogenic mechanism of IAS relating to DRB1*0406, analyzing the association between HLA DRB1 molecules, fragments of insulin peptide, and SH group compounds [4]. Our patient also carried DRB1*0406 (another allele was *0901). The coincidence of these two factors, HLA type and use of ALA, might have led to her development of IAS. DRB1*0406 is not a rare HLA molecule among Japanese. Considering the growing use of the health and nutrition supplements including ALA, there could be a future increase of IAS cases with the specific HLA type. Therefore, it will be important to pay attention for IAS as a cause of hypoglycemia in such cases.

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References

[1] M. Hakamata, M. Itoh, Y. Sudo, N. Miyata, Insulin autoimmune syndrome after the third therapy with methimazole, Intern. Med. 34 (1995) 410–412.

[2] D. Ziegler, F.A. Gries, Alpha-lipoic acid in the treatment of diabetic peripheral and cardiac autonomic neuropathy, Diabetes 46 (Suppl. 2) (1997) S62–S66.

[3] S.D. Wollin, P.J. Jones, Alpha-lipoic acid and cardiovascular disease, J. Nutr. 133 (2003) 3327–3330.

[4] S. Matsushita, K. Takahashi, M. Motoki, K. Komoriya, S. Ikagawa, Y. Nishimura, Allele specificity of structural requirement for peptides bound to HLA-DRB1*0405 and -DRB1*0406 complexes: implication for the HLA-associated susceptibility to methimazole-induced insulin autoimmune syndrome, J. Exp. Med. 180 (1994) 873–883.

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Mise au point thématique

Insulin autoimmune syndrome (IAS, Hirata disease)

Yasuko UCHIGATA, Yukimasa HIRATA

Although HLA and disease association has been studied for a tremendous number of diseases, only four diseases have been identified in which almost all patients have the same HLA antigen: B27 in 88% of ankylosing spondylitis (1), DR4 in 91% of patients with pemphigus vulgaris (2), DR2 in 100% of patients with narcolepsy (3), DRw52a in 100% of patients with primary sclerosing cholangitis (4). When the first patient with spontaneous hypoglycemia associated with the production of insulin autoantibodies, the so-called insulin autoimnmune syndrome (IAS), was reported in Japan by Hirata *et al.* (5) in 1970, no one could forecast that IAS was the fifth disease with such a strong HLA-association. The many questions raised by this first case, including its differential diagnosis from factitious hypoglycemia, the causes of this syndrome, the mechanisms to produce hypoglycemia in this syndrome and so on, raised doubt whether IAS was a "disease". Immediately after the first patient was diagnosed with IAS, several other patients with the same symptoms and findings were reported over five years (6-9). The strong association of IAS with HLA-DR4 (10) gave IAS a citizenship of "disease", which was named Hirata's disease. One hundred and ninety seven Japanese IAS patients have been registered from 1970 to 1992 (11) and a total of 244 Japanese IAS patients have been registered until the end of 1997. Besides the analysis of those reports, several studies concerning the causes of IAS and the hypoglycemia have been clarified by us.

Insulin autoimmune syndrome as the third leading cause of spontaneous hypoglycemia in Japan

To determine the further characteristics of IAS in Japanese, we performed two nationwide surveys for causes of cases with spontaneous hypoglycemia. Questionnaires were sent to 2094 hospitals with more than 200 beds; the first, from 1979 to 1981, the second, from 1985 to 1987. The first and the second surveys revealed the same results (12). Cases with hypoglycemia showed three main causes for the hypoglycemic attacks: insulinoma, extrapancreatic neoplasms and IAS. IAS was found to be the third leading cause of spontaneous hypoglycemia in Japan.

Diabetes Center, Tokyo Women's Medical University School of Medicine, 8-1 Kawada-cho, Shinjuku-ku, 162 Tokyo, Japan.

Correspondence and reprints: Y. UCHIGATA, at the address above. E-mail: uchigata@zengaku.twma.ac.jp

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Onset age and sex distribution, and duration of hypoglycemia of 244 Japanese IAS patients registered in Japan from 1970 to 1997

The records of 197 patients with IAS reported from 1970 to 1992 (11) and 47 patients from 1993 to 1997 were analyzed. The records of a total of 244 patients were obtained from nationwide hypoglycemia surveys, abstracts in local or national medical congress, and personal communications to us.

Age of onset and sex distribution of the 244 patients are shown in *(table I)*. The age distribution was wide at onset of IAS. The peak age of onset was 60-69 years for both sexes; there was no remarkable sex difference in the other age dis-

Table I. – Age at onset and sex distribution in Japanese IAS patients, 1970-1997.

Age at onset	Male (n)	Female (n)	Total
0-9	0	1	1
10-19	1	1	2
20-29	4	16	20
30-39	10	9	19
40-49	20	23	43
50-59	27	23	50
60-69	28	31	59
70-79	26	18	44
80-89	6	10	16
Total	122	132	244

Table II. – Diseases and drug exposure preceeding the development of IAS in Japanese (n = 144).

Drug	Disease	n
Methimazole	Graves' disease	49
α-mercaptopropionyl glycine	Chronic liver dysfunction	30
α-mercaptopropionyl glycine	Cataract	6
α-mercaptopropionyl glycine	Dermatitis	5
α-mercaptopropionyl glycine	Rheumatoid arthritis	2
Glutathione	Urticaria	8
Miscellaneous	–	44

tribution except in the 20-29 year group, in which 80% were female IAS patients. It seems that the 20-29 year group contained a larger number of female patients with Graves' disease than the others.

The duration of the transient and spontaneous hypoglycemia was shown to be less than 1 month in approximately 30% of the patients, more than 1 month and less than 3 months in 40% of the patients (11). A few of the patients have continued mild hypoglycemic attacks for more than 1 year.

The geographic distribution of IAS in Japan showed no characteristic pattern in the areas of residence of the patients.

Drug exposure ahead of development of IAS and associated diseases

As Hirata already noted in 1983, patients with Graves' disease who had received methimazole (MTZ) had a predisposition to develop IAS (13). In addition to methimazole (MTZ) for the treatment of Graves' disease, α-mercaptopropionyl glycine (MPG) for the treatment of chronic hepatitis, dermatitis, cataract and rheumatoid arthritis, and glutathione (GTT) for urticaria, which contained the sulfhydryl (SH) group, were proposed to be related to the development of IAS (11). Approximately 41% of Japanese IAS patients had received drugs with SH group *(table II)*. After such drugs were discontinued, the hypoglycemic attacks subsided. We have noted 4 IAS patients who developed IAS at the second treatment after an interruption of MTZ therapy, 1 IAS patient who developed the disease after the third challenge (after two interruptions of MTZ therapy), and 1 IAS patient at both the first and the second MTZ treatment. Another 3 patients redeveloped IAS at MPG challenge (14). Such evidence may support the breakdown of T cell immunotolerance in the circumstance described above.

Clinical features of IAS patients out of Japan

Although there have been 244 IAS patients reported from 1970 to 1997 in Japan, 10 IAS patients have been reported in East Asians excluding Japanese patients *(table IIIa)*. Nine of 10 IAS patients have associated with Graves' disease with the treatment of MTZ. Such patients were female and developed IAS at a younger age. HLA class II in 3 of them were analyzed, which was compatible with that in Japanese IAS patients and insulin autoantibodies were all polyclonal, as described later.

Table IIIa. – Clinical features in IAS cases in non Japanese East Asians.

Patient	Age	Sex	Disease/Drug	Race	Reference
1	52	M	Vasculitis	Chinese	29
2	48	F	Graves'/MTZ	Chinese	29
3	31	F	Graves'/MTZ	Korean	30
4	18	F	Graves'/MTZ	Chinese	31
5	67	F	–	Chinese	32
6	28	F	Graves'/MTZ	Chinese	32
7	26	F	Graves'/MTZ	Chinese	32
8	24	F	Graves'/MTZ	Chinese	32
9	21	F	Graves'/MTZ	Chinese	*
10	11	F	Hashimoto	Chinese	**

* unpublished, by Lin from Taiwan.
** unpublished, by Wacharasindhu from Thailand.

Table IIIb. – Clinical features in IAS cases out of Asia.

Patient	Age	Sex	Disease/Drug	Race	Reference
1	42	M	Pulmonary TB/INH	NS	7
2	58	F	RA/NS	White	33
3	43	F	–	Hispanic	33
4	3	M	–	NS	33
5	26	F	–	White	33
6	48	F	Brain damage	White	33
7	5	M	–	Hispanic	34
8	82	F	Lupus/hydralazine	White	35
9	61	F	RA/asthma/ penicillamine	White	29
10	55	F	Graves'/ carbimazole	White	36
11	44	M	Pulmonary TB/INH	Morocco	37
12	84	F	RA/pyritinol	White	38
13	74	M	Hypertention/ Hydralazine	Black	39
14	NS	NS	–	White	40
15	NS	NS	–	White	40
16	NS	NS	–	White	40
17	NS	NS	–	White	40
18	NS	NS	–	White	41
19	33	NS	RA/penicillamine	White	28
20	NS	NS	RA/pyritinol	White	42
21	NS	NS	–	White	43
22	63	M	–	White	44
23	57	F	–	White	45
24	5	M	–	White	46
25	57	F	Malaria/NS	White	*
26	45	F	–	White	21
27	48	F	–	White	47

NS: not stated; RA: rheumatoid arthritis; INH: isoniatid; TB: tuberculosis. —: no associated disease.
* unpublished, by Dozio from Italy.

So far, 27 IAS patients in Caucasians have been reported in the past 27 years *(table IIIb)*. MTZ for treatment of Graves' disease and penicillamine for treatment of rheumatoid arthritis were administered to 3 IAS patients, which all contained SH group. Insulin autoantibodies of 6 IAS patients so far examined were monoclonal as described later.

Insulin in the sera of the patients with IAS

The insulin in the sera of IAS patients was found to be native human insulin by HPLC analysis (15). *Figure 4* shows total extractable IRI and ^{125}I-insulin binding of the sera of patients with IAS. The IRI levels during hypoglycemic attacks were quite enormous (8). When hypoglycemia was severe, Scatchard analysis of the insulin antibodies showed that a high-affinity (k1)/low-capacity (b1) population of the antibodies was changed to relatively low affinity with very high binding capacity compared with the same population of antibodies in insulin-treated diabetic patient (16). When the attacks were relieved, the total IRI was decreased and the high-affinity (k1)/low-capacity (b1) population of antibodies showed a higher affinity constant and a lower binding capacity than those during the attacks (16).

A possible monoclonal insulin autoantibody which was of IgG1 subclass and has a very low affinity constant and a large binding capacity against human insulin was found to be directed at a determinant at the asparagine site on insulin B-chain (17). One of idiotypic antibodies againt the insulin autoantibody was found to mimic insulin action through the insulin receptor (18).

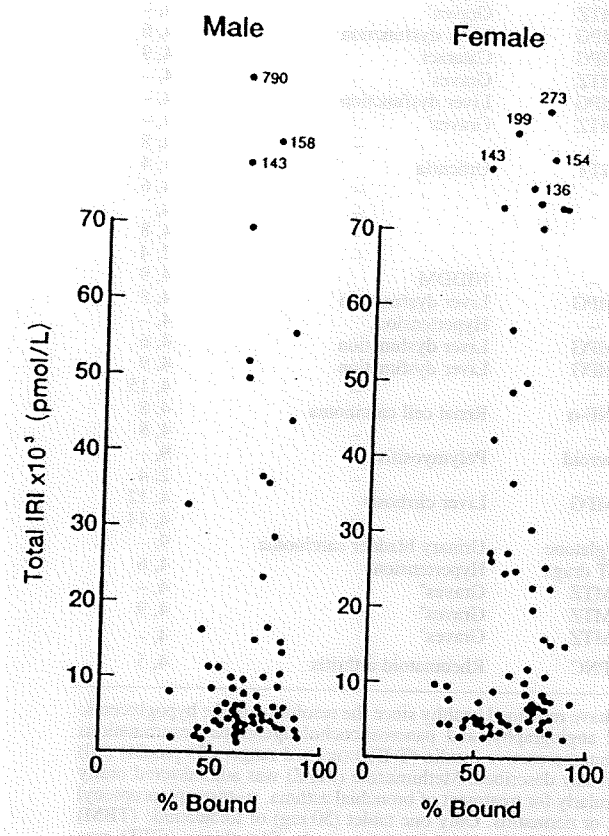

Fig. 4. – Total extractable immunoreactive insulin (IRI), and ^{125}I-insulin binding % of the sera of male and female patient, immediately after diagnosis of insulin autoimmune syndrome (IAS). At diagnosis of IAS, the peak of the hypoglycemic attacks had been passed. The normal range of total IRI and ^{125}I-insulin binding was < 71.8 pmo/l and < 5%, respectively.

Two group of IAS defined by clonality of insulin autoantibodies

The immunoglobulin class, the subclass and the light chain types of insulin autoantibodies were examined (19). All insulin autoantibodies belonged to the IgG group with various ratios of κ:λ light chains.

Insulin autoantibodies from IAS patients were classified as polyclonal or monoclonal on the basis of affinity curves for binding to human insulin (Scatchard analysis) and presence of solitary light chain. So far, 1 Japanese, 1 Norwegian, 1 Swiss, and 3 Italian IAS patients showed monoclonal autoantibodies to human insulin (20). Recently, we reported 1 Netherlander IAS patient (21). However, 50 of 51 Japanese IAS patients so far examined, 2 Korean, and 1 Chinese IAS patients showed polyclonal autoantibodies to human insulin *(table IV)* (20). Recently a Thai IAS patient was shown to possess a polyclonal insulin autoantibody. It is likely that the incidence of polyclonal IAS is relatively high among East Asians, whereas monoclonal IAS is more prevalent in Caucasians.

Critical amino acids for IAS polyclonal responder and importance of DR gene products in the presentation of human insulin antigen

As reported in the study of serological typing of 27 Japanese IAS patients, Cw4/B62/DR4 was a highly prevalent allelic combination (10). *Table IV* shows the summary of clinical characteristics of IAS polyclonal responders at onset of IAS which we examined. Japanese IAS polyclonal responders (except patients 45 and 49) possessed HLA-DR4/DQ3, whereas the remaining two (patients 45 and 49) possessed DR9/DQ3 and not DR4; the American white polyclonal responder possessed DR4/DQ3 *(table IV)*. Ninety-six percent (48 out of 50) of Japanese IAS patients had DR4 (Odds ratio, 39.9, $p<10\text{-}4$). DR9 was positive in 12 (24%) Japanese IAS patients, though, this was not significant compared with Japanese healthy controls (Odds ratio, 0.8, $p > 0.65$).

The 48 DR4-positive Japanese IAS polyclonal responders consisted of 42 DRB1*0406-positive, 5 DRB1*0403-positive, and 1 DRB1*0407-positive patients *(table V)*. All 48

Table IV. – Summary of clinical characteristics of IAS polyclonal responders at onset of IAS.

Ethnic background	Patient no	Age (years)	Sex	T-IRI (pmol/l × 10^3)	^{125}I-insulin binding (%)	Drug	Associated disease	HLA-DR
Japanese	1	26	F	114.3	69	MTZ	Graves'	4, –
Japanese	2	52	F	54.5	73	GTG	Bronchial asthma	4, 13
Japanese	3	47	M	23.4	79	–	–	4, 9
Japanese	4	58	M	0.8	48	–	–	4, –
Japanese	5	54	F	60.0	70	MTZ	Graves'	4, –
Japanese	6	36	M	8.2	59	–	–	4, 9
Japanese	7	45	F	7.2	32	MTZ	Graves', Hashimoto's, IgA nephropathy	4, 8
Japanese	8	62	M	12.6	69	–	–	4, 9
Japanese	9	54	M	4.4	81	MPG	Liver dysfunction	2, 4
Japanese	10	61	F	5.1	72	MPG	Cataract	4, 9
Japanese	11	44	M	3.0	70	MPG	Dermatitis	2, 4
Japanese	12	39	F	2.7	69	MTZ	Graves'	4, –
Japanese	13	69	M	12.3	65	–	–	4, 6
Japanese	14	57	F	2.8	51	–	–	4, 9
Japanese	15	68	F	11.7	68	MPG	Liver dysfunction	4, 8
Japanese	16	48	M	11.1	81	MTZ	Graves', drug-induced arthritis	4, –
Japanese	17	64	M	4.6	82	MPG	Dermatitis	4, 13
Japanese	18	58	F	12.0	81	–	–	4, 12
Japanese	19	49	M	11.9	48	MPG	Liver dysfunction	4, 12
Japanese	20	69	F	167.2	67	TBM	NIDDM	4, –
Japanese	21	55	M	0.9	48	MPG	Liver dysfunction	4, 8
Japanese	22	53	F	35.0	55	–	–	4, 9
Japanese	23	69	M	47.0	65	MTZ	Graves'	4, 8
Japanese	24	36	F	192.5	79	MTZ	Graves'	2, 4
Japanese	25	43	F	23.0	57	MTZ	Graves'	4, –
Japanese	26	68	M	5.4	64	MPG	Liver dysfunction	4, 6
Japanese	27	66	F	21.0	57	MPG	Cataract	4, 9
Japanese	28	49	F	18.0	83	MTZ	Graves'	4, –
Japanese	29	54	M	3.4	67	MPG	Liver dysfunction	4, –
Japanese	30	70	F	46.8	94	MTZ	Graves'	4, –
Japanese	31	67	F	120.0	80	–	–	4, 8
Japanese	32	50	F	1.7	64	GTT	Urticaria	1, 4
Japanese	33	52	M	13.3	38	–	–	4, 6
Japanese	34	74	M	5.2	78	–	–	4, –
Japanese	35	54	M	3.0	76	–	–	4, 8
Japanese	36	79	M	3.1	70	–	–	2, 4
Japanese	37	49	F	1.4	88	–	NIDDM	4, 9
Japanese	38	59	M	24.3	69	MPG	Liver dysfunction	4, 8
Japanese	39	66	M	6.2	91	–	Hypertension	4, –
Japanese	40	84	F	5.0	73	MPG	Liver dysfunction	4, 6
Japanese	41	42	F	2.5	24	MPG	Liver dysfunction	4, 9
Japanese	42	71	F	5.1	67	–	–	4, 15
Japanese	43	70	F	4.5	84	INF-α	Renal cell carcinoma	4, 8
Japanese	44	65	M	1.1	50	–	–	4, 8
Japanese	45	64	M	9.3	48	Steroid	Polymyositis	9, –
Japanese	46	49	F	5.4	75	–	–	2, 4
Japanese	47	71	M	3.0	68	MPG	Liver cirrhosis	4, 12
Japanese	48	81	M	1.4	92	–	–	4, 14
Japanese	49	77	M	0.7	64	Aceglatone	Urinary bladder carcinoma	9, –
Japanese	50	71	F	7.4	80	AHT drugs	Hypertension	4, 6
Korean	1	61	F	12.0	90	MTZ	Graves'	4, –
Korean	2	31	F	8.9	62	MTZ	Graves'	4, 9
Chinese	1	18	F	36.4	82	MTZ	Graves'	4, –
White American	1	61	F	4.8	1:64*	PNC	Rheumatoid arthritis	4, 5

Hypoglycemic attacks occured - 6 weeks after drug administration. All of the patients have remained healthy since the resolution of the hypoglycemic attacks (21). Abdominal computed tomography, abdominal ultrasound, abdominal angiography, and pancreaticocholangiography examinations performed in six patients failed to reveal the presence of a pancreatic tumor. Total immunoreactive insulin (T-IRI) (normal range, <35 pmol/l) (10) and ^{125}I-labeled human insulin binding (normal range, <5%) were measured as previously described. Methimazole (MTZ) was administered orally for treatment of Graves' disease. Gold thioglucose (GTG) was administered intramuscularly for treatment of bronchial asthma. α–mercaptopropionyl glycine (MPG) was administered orally for treatment of liver dysfunction, cataracts, or dermatitis. Only one tablet (50 mg) of tolbutamide (TBM) was administered orally for treatment of non-insulin dependent diabetes (NIDDM) before the hypoglycemic attack. Glutathione (GTT) was administered intravenously for treatment of urticaria. Interferon-α (IFN-α) was administered intravenously for treatment of renal cell carcinoma (27). The anticancer drug aceglatone was administered orally for treatment of urinary bladder carcinoma. Japanese patient 50 was taking antihypertensive (AHT) drugs when IAS developped. Penicillamine (PNC) was administered orally for treatment of rheumatoid arthritis. Japanese patients 33, 36, 42, 46, and 48 possessed the DRB1*0403 allele. Japanese patient 37 and the white American patient possessed the DRB1*0407 allele. The remaining patients, with the exception of Japanese patients 45 and 49 (DR9 homozygotes), possessed the DRB1*0406 allele. * 1: 64 was expressed as positive.

Table V. – Incidence of DRB1 alleles, Glu^{74} in DR β1-chain and DQB1 alleles in Japanese IAS polyclonal responders and control subjects.

	IAS patients	Control	OR (95% confidence interval)	DRB1 chain amino acid residue		
				37	74	86
n	50	106	–	–	–	–
DRB1 allele						
DR4	48 (96)	40 (38)	39.6 (9.12-171)	–	–	–
DR9	12 (24)	29 (27)	0.8 (0.39-1.82)	Asn	Glu	Gly
DRB1*0406	42 (84)	9 (8)	56.6 (20.4-156)	Ser	Glu	Val
DRB1*0403	5 (10)	7 (7)	1.6 (0.47-5.22)	Tyr	Glu	Val
DRB1*0407	1 (2)	2 (2)	1.1 (0.09-12.0)	Tyr	Glu	Gly
Glu^{74} in β–chain	50 (100)	70 (66)	52.3 (6.95-393)	–	–	–
DQB1 allele						
DQA1*0301	50 (100)	74 (70)	44.1 (5.84-332)	–	–	–
DQA1*0302	48 (96)	26 (25)	73.8 (16.8-325)	–	–	–
DQA1*0301/DQB1*0302	48 (96)	23 (22)	86.6 (18.8-380)	–	–	–

Data are *n* (%) or OR (95% confidence interval).

DR4-positive Japanese IAS polyclonal responders possessed DQA1*0301/DQB1*0302 regardless of the differences in DR4 alleles. The two Korean and the Chinese IAS polyclonal responders were also positive for DRB1*0406/DQA1*0301/DQB1*0302. The phenotype of the American polyclonal responder was DRB1*0407/DQA1*0301/DQB1*0301. Thus, the DR4-positive IAS polyclonal responders possessed DRB1*0406, DRB1*0403, or DRB1*0407 for DR4 alleles, and DQA1*0301/DQB10302 or DQA1*0301/DQB1*0301 for DQ3 alleles.

Among DRB1*0406 individuals, incidence of B62/Cw4 was compared in Japanese IAS polyclonal responders and Japanese healthy controls. Sixty-seven percent (28 out of 42) of Japanese IAS polyclonal responders had B62/Cw4, while 56 percent (5 out of 9) of controls possessed it (Odds ratio, 1.6; 95% confidence interval, 0.37-6.91). This indicates that the class I alleles are less important in the susceptibility to the disease.

The differences in DQB1 alleles encoding DQ3 among the IAS polyclonal responders suggest that DQ α and DQ β chains are not important in the development of IAS. We showed that T cells from polyclonal IAS patients with DRB1*0406/DQA1*0301/DQB1*0302 alleles proliferated in the presence of autologous antigen-presenting cells that had been exposed to 40 μM human insulin (22). The proliferative response of T cells was completely blocked by anti-HLA-DR but not by anti-HLA-DQ monoclonal antibodies *(fig. 2)* (23). Moreover, experiments with DRB1*0406 transfectants supported the view that DR gene products participate in the presentation of human insulin antigens *(table VI)* (23).

The HLA-DR β1 chains encoded by DRB1*0406, DRB1*0403, and DRB1*0407 share a sequence motif (Leu-Leu-Glu-Gln-Arg-Arg-Ala-Glu) that spans the amino acid residues 67-74 of the third hypervariable region. The two DR9/DQ3 Japanese IAS polyclonal responders (patients 45 and 49) were DRB1*0901/ DQA1*0301/ DQB1*0303 homozygous. The products of DRB1*0406,

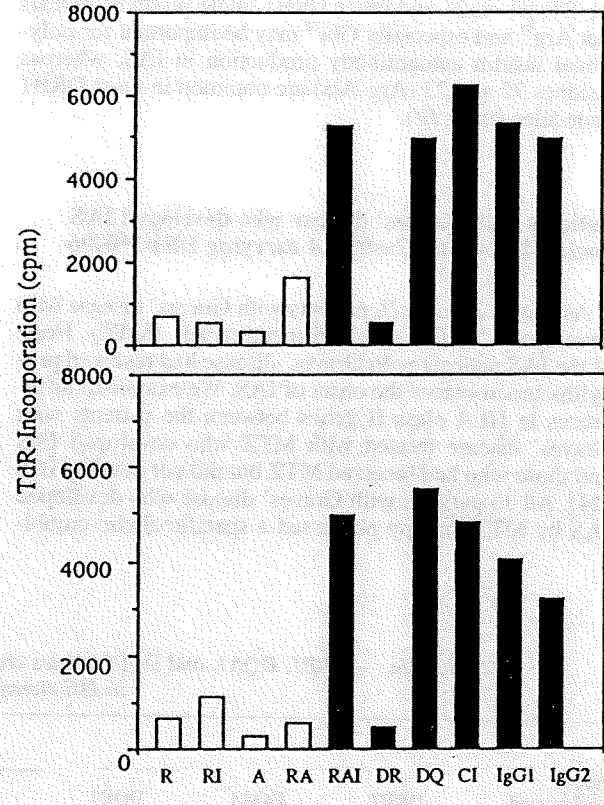

Fig. 2. – T cell responses (top from donor MI and bottom from donor SO) to human insulin blocked by anti-HLA DR monoclonal antibody (mAb). Results represent the mean of triplicate (SD<15% of the mean). R, enriched T cells; RI, R+ human insulin; A, antigen-presenting cells; DR, R + A + I + anti-HLA-DRmAb; DQ, R + A + I +anti-HLA-DQmAB; CI, R + A + I +anti-HLA class I mAb; IgG1, R + A + I + mouse IgG1; IgG2a, R + A + I + mouse IgG2a.

*Table VI. – HLA-DRB1*0406-transfected L cells can present human insulin to enriched T cells from a healthy donor or an IAS patient with-DRB1*0406 in the presence of DTT[a].*

	DRB1*	[3H]thymidine Incorporation (CPM) R Only	R + L	R + L + DTT	R + L + HI	R + L + HI + DTT
IAS patients						
IS	0406	1834 ± 23	905 ± 79	1554 ± 138	16,267 ± 306	12,692 ± 374
SM	0406	2396 ± 199	5167 ± 263	6104 ± 413	ND	15,218 ± 822
Healthy donors						
JJ	0406	247 ± 141	3711 ± 41	3493 ± 572	7280 ± 1,874	11,854 ± 538
KA	0406	208 ± 25	1618 ± 465	1518 ± 391	5727 ± 607	7492 ± 248
NA	0405	328 ± 25	457 ± 4	ND	506 ± 46	ND
ME	0405	1563 ± 416	2819 ± 251	1309 ± 15	572 ± 32	630 ± 59

[a] The 5×10^3 irradiated (80 Gy) L cell transfectants (L) were incubated with or without 40 μ M human insulin (HI) in medium containing 2 mM DTT and cultured for 6 days with 5×10^4 enriched T cells (R, responder cells) [3H]thymidine incorporation of L cells alone were 3300 ± 97 for DRB1*0406 transfectant and 1586 ± 94 for DRB1*0405 transfectant, respectively.

DRB1*0403, and DRB1*0901 share the sequence motif Arg-Arg-Ala-Glu, corresponding to amino acid residues 71-74 of the DR β1chain. Comparison of this region of the DR β1 chain and other DRB1 allele products reveals that Arg^{71} and especially Glu^{74} may be important for polyclonal insulin autoantibody production in IAS, whereas residues 72 and 73 (Arg-Ala) are common in most DRB1 molecules *(table IV)*.

Patients with Graves' disease who developed IAS possess HLA-B62/Cw4/DR4 carrying DRB1*0406

As shown in *table II*, patients with Graves' disease who developed IAS had received methimazole (MTZ). None of the IAS patients with Graves' disease had received propylthiouracil before the onset of IAS. We examined differences in HLA class II genes between the patients with Graves' disease treated with MTZ who developed IAS and those who had received MTZ but did not develop IAS (24). All 13 patients with Graves' disease who developed IAS by MTZ therapy possessed a specific allelic combination, B62/Cw4/DR4 carrying DRB1*0406, whereas only one of 50 Graves' disease patients without IAS had B62/Cw4/DR4 and those 50 did not possess DRB1*0406. It is highly likely that patients with Graves' disease develop IAS via treatment with MTZ when their B62/Cw4/DR4 carry DRB1*0406.

Different amino acids for IAS monoclonal responder

The IAS monoclonal responder group consisted of 6 patients: 1 Japanese, 1 Norwegian, 1 Swiss, and 3 Italians *(table VII)*. Three of the 6 IAS monoclonal responders were DR4-positive and their class II phenotypes were DRB1*0405/DQA1*0301/DQB1*0401, DRB1*0401/DQA1*0301/DQB1*0301, and DRB1*0402/DQA1*0301/DQB1*0301 *(table VII)*. The remaining three IAS monoclonal responders had non-DR4 and non-DR9 phenotypes. The IAS monoclonal responders did not express Glu^{74} but Ala^{74}, and Asp^{57} and Gly^{86} on the DRB1 chain were shared by the six monoclonal responders. This finding suggests that the mechanism of monoclonal insu-

Table VIIa. – DRB1, DQA1, and DQB1 alleles and comparison of amino acid residues in the DRβ1 chain in IAS monoclonal responders.

Ethnic background	DRB1	DQA1	DQB1	DRB1 chain amino acid residue 37	47	57	74	86
Japanese	0405/0803	0301/0103	0401/0601	Tyr	Tyr	Ser/*Asp*	*Ala*/Leu	Gly/Val
Norwegian	0401/–	0301/–	0301/–	Tyr	Tyr	*Asp*	*Ala*	Gly
Swiss	0101/1601	0101/0102	0501/0502	Ser	Tyr	*Asp*	*Ala*	Gly
Italian	1501/1502	0102/0103	0601/0602	Ser	Phe	*Asp*	*Ala*	Gly/Val
Italian	0701/1501	0201/0102	0201/0602	Ser/Phe	Phe/Tyr	Val/*Asp*	*Ala*/Gln	Gly/Val
Italian	0402/1101	0301/0501	0301/0302	Tyr	Phe/Tyr	*Asp*	*Ala*	Gly
Dutch	0404/0301	0301/0501	0302/0201	Tyr/Ser	Tyr/Phe	*Asp*	*Ala*/Arg	Val

lin autoantibody production in IAS is different from that of the production of polyclonal antibodies. One appropriate question that our data raise is whether antigen presentation efficiency of insulin peptides in patients with Ala^{74} in the DR β1 chain is indeed reduced compared with that in Glu^{74}-positive patients.

Possible role of the specific amino acids on the DR β chain in IAS pathogenesis

Based on the findings described above, we conclude that 1) DR4 is the dominant phenotype in terms of susceptibility to IAS, 2) DRB1*0406 is associated with the highest risk for the susceptibility to IAS, and 3) Glu^{74} in the DR4 β1 chain is essential for polyclonal IAA production in IAS. 4) Ser^{37} in the DR4 β1 chain may have a significant additive effect on polyclonal autoantibody production *(table V)*. We describe that DRB1*0406 is an allele for the susceptibility to IAS with polyclonal IAA.

The three-dimensional structure of the HLA class II DR1 molecules determined by X-ray crystallography has shown an open-ended groove, in which the peptides processed by antigen-presenting cells are bound as straight extended chains (25) and an anchoring peptide side chain of the processed peptides was found to fit in a prominent nonpolar pocket near one end of the binding groove (25). Matsushita *et al.* (26) reported that peptide of human insulin A-chain (^{8}TSICSLYQLE^{17}) was shown to bind specifically to DRB1*0406 using its ^{10}IxxLxxQ^{15} motif. The second anchor residue was reported to exhibit allele specificity in binding, especially with the amino acid residue 74 of DRB1 chain (26). However, the interaction of L (Leu) of insulin peptide with ^{74}Glu in the DR4 β chain has remained questionable because Leu was a hydrophobic residue and Glu was an acidic residue. Other portions of human insulin may be a candidate for presentation and recognition for T cells in IAS (in preparation).

Although there are controversial points in the antigen-HLA-DRB1*0406 molecule-T cell receptor interaction, reducing compounds such as MTZ, MPG, or glutathione may cleave disulfide bonds of natural human insulin in vivo and expose self-antigens such as insulin-derived peptides to DRB1*0406 molecules on antigen-presenting cells, resulting in insulin specific proliferating T cells. As mentioned previously, T-cell recognition of human insulin in the context of DRB1*0406 molecules showed the highest risk for the susceptibility to IAS in the polyclonal IAS responders, whereas T-cell recognition in the context of DRB1*0403 or DRB1*0407 did not. When human insulin-derived peptides were tested (for example, amino acids 8-17 of A chain), they were indeed recognized by T cells in the context of DRB1*0403 or DRB1*0407 (in preparation).

Because polyclonal IAS patients exhibit typical polyclonal immunoglobulin G response to human insulin, the response may be an antigen-driven immune one with T-cell help. Accordingly, typical HLA-and peptide-restricted recognition may contribute to the initiating event in the pathogenesis of IAS, in which Glu^{74} may act as the primary residue in the peptide-binding interaction.

Natural history of IAS

Table VIII shows the clinical course and IAS treatments. More than 80% of Japanese IAS patients had spontaneous remission *(table VIII)*. Spontaneous remission may have developed in less than 3 months. Some patients needed medication to stop the persistent hypoglycemic attacks; steroids, azathioprine, or 6-mercaptopurine. In five patients, plasmapheresis was performed to wash out insulin autoantibodies from sera. In the early 1970s, partial pancreas excision surgery was performed in 6 patients under misdiagnosis of insulinoma. Hyperplasia of the islet B cells in the excised part of the pancreas was reported in some IAS patients (5, 6).

The current recommended management is to give small meals divided into six or more and to avoid sweets except at the time of hypoglycemic attacks. Alpha-glucosidase inhibitors may sometimes be helpful in decreasing immunoreactive insulin in the sera after taking a meal (21).

A novel concept of type VII hypersensitivity introduced by insulin autoimmune syndrome (Hirata' s disease)

Disorders resulting from aberrant, excessive or uncontrolled immune responses are called hypersensitivity diseases. Hypersensitivity diseases that are supposed to be due to immune responses against self antigens are called autoimmune diseases.

Based on the principal criterion of the type of immune responses that leads to tissue injury, the conventional classification consists of type I (immediate hypersensitivity), type II (antibody-mediated), type III (immune complex-mediated), and type IV (T cell-mediated). Two new types V and VI, have recently been derived from type II and proposed as "antibody-mediated hyperfunction of the target tissues" in which Graves' disease is representative and "antibody-dependent cell-mediated cytotoxicity (ADCC)", respecti-

Table VIII. – Disease course and treatment in 226 Japanese IAS patients.

	Male 112	Female 114	Total 226
Spontaneous remission	97	92	189 (83.6 %)
Treatment			
Steroids	6	18	24 (10.6 %)
Pancreatic surgery	3	3	6 (2.6 %)
Plasmapheresis	5	0	5 (2.2 %)
Azathioprine	1	0	1 (0.4 %)
6-Mercaptopurine	0	1	1 (0.4 %)

vely. We propose a new concept of type VII hypersensitivity defined as immunologic diseases which are induced by the release of self-antigens from the bound autoantibodies in serum. The self-antigens in type VII hypersensitivity are supposed to be located in the liquid phase, different from the self-antigens on the cell membranes in type II and IV hypersensitivities. IAS is a representative disease in type VII hypersensitivity (14).

References

1. SCHLOSSTEIN L, TERASAKI PI, BLUESTONE R *et al.* High association of an HL-A antigen B27 with ankylosing spondylitis. *N Engl J Med*, 1973; *288:* 704-706.
2. PARK MS, TERASAKI PI, AHMED AR *et al.* HLA-DRw4 in 91% of jewish pempemphigus vulgaris patients. *Lancet*, 1979; *2:* 441-443.
3. JUJI T, SATAKE M, HONDA Y *et al.*: HLA antigens in Japanese patients with narcolepsy. *Tissue Antigens*, 1984; *24:* 316-319.
4. PROCHAZKA EJ, TERASAKI PI, PARK MS *et al.*: Association of primary sclerosing cholangitis with HLA-DRw52a. *N Engl J Med*, 1990; *322:* 1842-1844.
5. HIRATA Y, ISHIZU H, OUCHI N *et al.*: Insulin autoimmunity in a case of spontancous hypoglycemia. *J Jpn Diabetes Soc*, 1970; *13:* 312-320.
6. HIRATA Y, ARIMICHI M. Insulin autoimmune syndrome-the second case. *J Jpn Diabetes Soc*, 1972; *15:* 187-192.
7. FOLLING J, NORMAN N. Hyperglycemia, hypoglycemic attacks and production of anti-insulin autoantibodies without previous known immunization. Immunological and functional studies in a patient. *Diabetes*, 1972; *21:* 814-826.
8. HIRATA Y, TOMINAGA M, ITO J *et al.*: Spontaneous hypoglycemia with insulin autoimmunity in Graves'disease. *Ann Intern Med*, 1974; *81:* 214-218.
9. ONEDA A, MATSUDA K, SATO M *et al.*: Hypoglycemia due to apparent autoantibodies to insulin. Characterization of insulin-binding protein. *Diabetes*, 1974; *23:* 41-50.
10. UCHIGATA Y, KUWATA S, TOKUNAGA K *et al.*: Strong association of insulin autoimmune syndrome with HLA-DR4. *Lancet*, 1992; *339:* 393-394.
11. UCHIGATA Y, EGUCHI Y, TAKAYAMA-HASUMI S *et al.*: Insulin autoimmune syndrome (Hirata disease): Clinical features and epidemiology in Japan. *Diabetes Res Clin Pract*, 1994; *22:* 89-94.
12. TAKAYAMA-HASUMI S, EGUCHI Y, SATO A *et al.*: Insulin autoimmune syndrome is the third leading cause of spontaneous hypoglycemic attacks in Japan. *Diabetes Res Clin Pract*, 1990; *10:* 211-214.
13. HIRATA Y. Methimazole and insulin autoimmune syndrome with hypoglycemia. *Lancet*, 1983; *ii:* 1037-1038.
14. UCHIGATA Y, HIRATA Y, OMORI Y. A nobel concept of type VII hypersensitivity induced by insulin autoimmune syndrome (Hirata's disease). *Autoimmunity*, 1995; *20:* 207-208.
15. WASADA T, EGUCHI Y, TAKAYAMA-HASUMI S *et al.* Reverse phase high performance loquid chromatographic analysis of circulating insulin in the insulin sutoimmune syndrome. *J Clin Endocrinol Metab*, 1988; *66:* 153-158.
16. EGUCHI Y, UCHIGATA Y, YAO K *et al.*: Longitudinal changes of serum insulin concentration and insulin antibody features in persistant insulin autoimmune syndrome (Hirata's disease). *Autoimmunity*, 1994; *19:* 279-284.
17. UCHIGATA Y, YAO K, TAKAYAMA-HASUMI S *et al.*: Human monoclonal IgG1 insulin autoantybody from insulin autoimmune syndrome directed at determinant at asparagine site on insulin B-chain. *Diabetes*, 1989; *38:* 663-666.
18. UCHIGATA Y, TAKAYAMA-HASUMI S, KAWANISHI K *et al.*: Inducement of antibody that mimics insulin action on insulin receptor by insulin autoantibody directed at determinant at asparagine site on human insulin B chain. *Diabetes*, 1991; *40:* 966-970.
19. UCHIGATA Y, EGUCHI Y, TAKAYAMA-HASUMI S *et al.*: The immunoglobulin class, the subclass and the ratio of k:É...light chain of autoantibodies to human insulin in insulin autoimmune syndrome. *Autoimmunity*, 1983; *3:* 289-297.
20. UCHIGATA Y, TOKUNAGA K, NEPOM G *et al.*: Differential immunologenetic determinants of polyclonal insulin autoimmune syndrome (Hirata's disease) and monoclonal insulin autoimmune syndrome. *Diabetes*, 1995; *44:* 1227-1232.
21. SCHLEMPER RJ, UCHIGATA Y, FROLICH M *et al.*: Recurrent hypoglycemia caused by the insulin autoimmune syndrome: the first dutch case. *Neth J Med*, 1996; *48:* 188-192.
22. UCHIGATA Y, OMORI Y, NIEDA M *et al.*: HLA-DR4 genotype and insulin-processing in insulin autoimmune syndrome. *Lancet*, 1992; *340:* 1467.
23. ITO Y, NIEDA M, UCHIGATA Y *et al.*: Recognition of human insulin in the context of HLA-DRB1*0406 products by T cells of insulin autoimmune syndrome patients and healthy donors. *J Immunol*, 1993; *15:* 5770-5776.
24. UCHIGATA Y, KUWATA S, TSUSHIMA T *et al.*: Patients with Graves' disease who developed insulin autoimmune syndrome (Hirata disease) possess HLA-Bw62/Cw4/DR4 carrying DRB1*0406. *J Clin Endocrinol Metab*, 1993; *77:* 249-254.
25. BROWN JHG, JARDETZKY TS, GORGA JC *et al.*: Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature*, 1993; *364:* 33-39.
26. MATSUSHITA S, TAKAHASHI K, MOTOKI M *et al.*: Allele specificity of structural requirement for peptides bound to HLA-DRB1*0405 and DRB1*0406 complexes: implication for the HLA-associated susceptibility to methimazole-insuced insulin autoimmune syndrome. *J Exp Med*, 1994; *180:* 873-883.
27. AKAI H, SUZUKI S, HIRAI S *et al.*: A case of insulin autoimmune syndrome developed during alpha-interferon therapy for renal cell cancer (Japanese). *Diabetes J*, 1994; *22:* 17-20.
28. BENSON EB, HEALEY LA, BARRON EJ *et al.*: Insulin antibodies in patients receiving penicillamine. *Am J Med*, 1985; *78:* 857-861.
29. BENSON EB, HO P, WANG C *et al.*: Insulin autoimmunity as a cause of hypoglycemia. *Arch Intern Med*, 1984; *144:* 2351-2354.
30. CHO BY, LEE HK, KOH CS *et al.*: Spontaneous hypoglycmeia and insulin autoantibodies in patients with Graves' disease. *Diabetes Res Clin Prac*, 1987; *3:* 119-124.
31. LEE Y-J, SHIN S-J, TORNG J-K *et al.*: Insulin autoimmune syndrome in a methimazole-treated Graves' disease with polyclonal anti-insulin autoantibodies. *J Formosa Med Assoc*, 1987; *867:* 164-170.
32. CHEN C-H, HUANG M-J , HUANG B-Y *et al.*: Insulin autoimmune syndrome as a cause of hypoglycemia. Report of four cases. *Cang Guang Med*, 1990; *42.*
33. GOLDMAN J, BALDWIN D, RUBENSTEIN A, KLINK DD: Characterization of circulating insulin and proinsulin-binding antibodies in autoimmune hypoglycemia. *J Clin Invest*, 1979; 1050-1059.
34. ROVIRA A, VALVERDE I, ESCORIHUELA R *et al.*: Autoimmunity to insulin in a child with hypoglycemia. *Acta Pediatr Scand*, 1982; *71:* 343-345.

35. BLACKSHEAR P, RHIL D, RONER HE *et al.*: Reactive hypoglycemia and insulin autoantibodies in drug-induced lupus erythematosus. *Ann Intern Med*, 1983; *99:* 182-184.
36. SKLENAR I, WILKIN TJ, DIAZ J-L *et al.*: Spontenous hypoglycemia associated with autoimmunity specific to human insulin. *Diabetes Care*, 1987; *10:* 152-159.
37. TENN G, ERYSSENLEIN V, MELLINGHOFF HU *et al.*: Clinical and biochemical aspects of the insulin autoimmune syndrome (IAIS). *Klin Wochenschr*, 1986; *64:* 929-934.
38. ARCHAMBEAUD-MOUVEROUX F, CANIVET B, FRESSINAUD C *et al.*: Hypoglycemie autoimmune; responsabilite du pyritinol? *Presse Med*, 1988; *17:* 1733-1737.
39. BURTH HB, CLEMENT S, SOKOL MS *et al.*: Reactive hypoglycemic coma due to insulin autoimmune syndrome: Case report and literature review. *Am J Med*, 1992; *92:* 681-685.
40. DOZIO N, SODOYEZ-GOFFAUX F, KOCH M: Are insulin autoantibodies different from exgenous insulin inuced antibodies ? *Diabetologia*, 1987; *30 (Supp):* 515A.
41. ANDERSON JH, BLACKARD WG, GOLDMAN J *et al.*: Diabetes and hypoglycemia due to insulin antibodies. *Am J Med*, 1978; *64:* 868-873.
42. FAGUER DE MOUSTIER B, BURGARD M, BIOTARD C *et al.*: Syndrome hypoglycemique auto-immun iduit par le pyritinol. *Diabete Metab*, 1988; *14:* 423-429.
43. DISCHER T, SEIPKE G, FRIEDMANN E *et al.*: Recurrent hypoglycemia in the insulin autoimmune syndrome. *Dtsch Med Wochenschr*, 1990; *115:*1951-1955.
44. SLUITER WJ, MARRINK J, HOUWEN B: Monoclonal gammopathy with an insulin binding IgG(k) M-component, associated with severe hypoglycemia. *Br J Heamotol*, 1986; *62:* 679-687.
45. SCAVINI M, DOZIO N, SARTORI S *et al.*: Effect of treatment on insulin bioavailability and ^{123}I insulin biodistribution in insulin immune hypoglycemia. *Diabetologia*, 1992; *35 (Suppl):* 187A.
46. MESCHI F, DOZIO M, BOGNETTI E *et al.*: An unusual case of recurrent hypoglycemia; 10-year follow up of a child with insulin auto-immunity. *Eur J Pediatr*, 1992; *151:* 32-34.
47. ARNQVIST H, HALBAN PA, MATHIESEN UL *et al.*: Hypoglycemia caused by atypical insulin antibodies in a patient with benign monoclonal gammopathy. *J Intern Med*, 1993; *234:* 421-427.

ELSEVIER

Worldwide Differences in the Incidence of Insulin Autoimmune Syndrome (Hirata Disease) with Respect to the Evolution of HLA-DR4 Alleles

Yasuko Uchigata, Yukimasa Hirata, Yasue Omori, Yasuhiko Iwamoto, and Katsushi Tokunaga

ABSTRACT: The relationship between the geographic distribution of susceptibility genes to insulin autoimmune syndrome (IAS) and the incidence of insulin autoimmune syndrome was investigated in order to examine the distribution of the genetic background to susceptibility to certain diseases. The HLA-DR4 allele, DRB1*0406, is associated with increased susceptibility to IAS among Japanese, while the DRB1*0403 and DRB1*0407 alleles are not (the odds ratio of which are 1.6 and1.1, respectively). The worldwide geographic distribution of the three DR*04 alleles showed that the distribution of DRB1*0403 encompassed that of DRB1*0406 and DRB1*0407. Taken together with the findings that Glu at position 74 in the DRB1 molecule is shared by the three DRB1*04 alleles, there are only a few differences between the DRB1 molecule-nucleotide sequences of DRB1*0403, DRB1*0406 and DRB1*0407, and that all the three DRB1*04 alleles are carried by the same class II haplotype, DQA1*0301/DQB1*0302, it may be considered that DRB1*0403 is the ancestral allele of DRB1*0406 and DRB1*0407. Therefore, populations with a higher prevalence of DRB1*0406 have a higher risk of developing IAS. The extremely low prevalence of IAS among Caucasians can be explained by the low prevalence of DRB1*0406 in this population. This is a good example of the association between the predisposition to risk of development of certain diseases and the evolution of susceptibility genes. *Human Immunology 61, 154–157 (2000).*

KEYWORDS: insulin autoimmune syndrome; HLA-DR4 alleles; disease susceptibility; hypoglycemia; incidence difference

INTRODUCTION

The HLA (human leukocyte antigen) system is a highly polymorphic genetic system in humans and consists of several closely linked genes. Because of the high degree of polymorphism in this system, analysis of the HLA loci represents a valuable tool to trace the migration of ancient human populations [1]. Actually, the genetic study of populations distributed throughout the world supports the speculation that migrants out of East and central Asia have colonized the New World, Japan, and Polynesian and Micronesian Islands [2, 3]. Furthermore, the HLA system is also used when examining the genetic susceptibility of a number of diseases. Some of the diseases are strongly related to specific HLA class I or class II molecules [4–7]. Such characteristics of the HLA system suggest that the evolution of genes may be associated with the susceptibility to diseases.

The insulin autoimmune syndrome (IAS, Hirata disease) is characterized by a combination of fasting and sometimes postprandial hypoglycemia, high serum concentrations of total immunoreactive insulin and presence in the serum of polyclonal autoantibodies against native human insulin (IAAs) [8]. Predisposition to IAS is significantly influenced by the ethnic background of the subject, with East Asians exhibiting a higher incidence than caucasians. Since the report of the first case was reported by Hirata et al. in 1970, 244 patients with IAS had been reported in Japan by the end of 1997 [9], while only 26 patients had been reported from various caucasian populations by the end of the same year [9].

From the Diabetes Center, Tokyo Women's Medical University School of Medicine (Y.U, Y.H, Y.O., Y.I.), and Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan (K.T.).

Address reprint requests to: Yasuko Uchigata, M.D., Ph.D., Diabetes Center, Tokyo Women's Medical University School of Medicine, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162, Japan.

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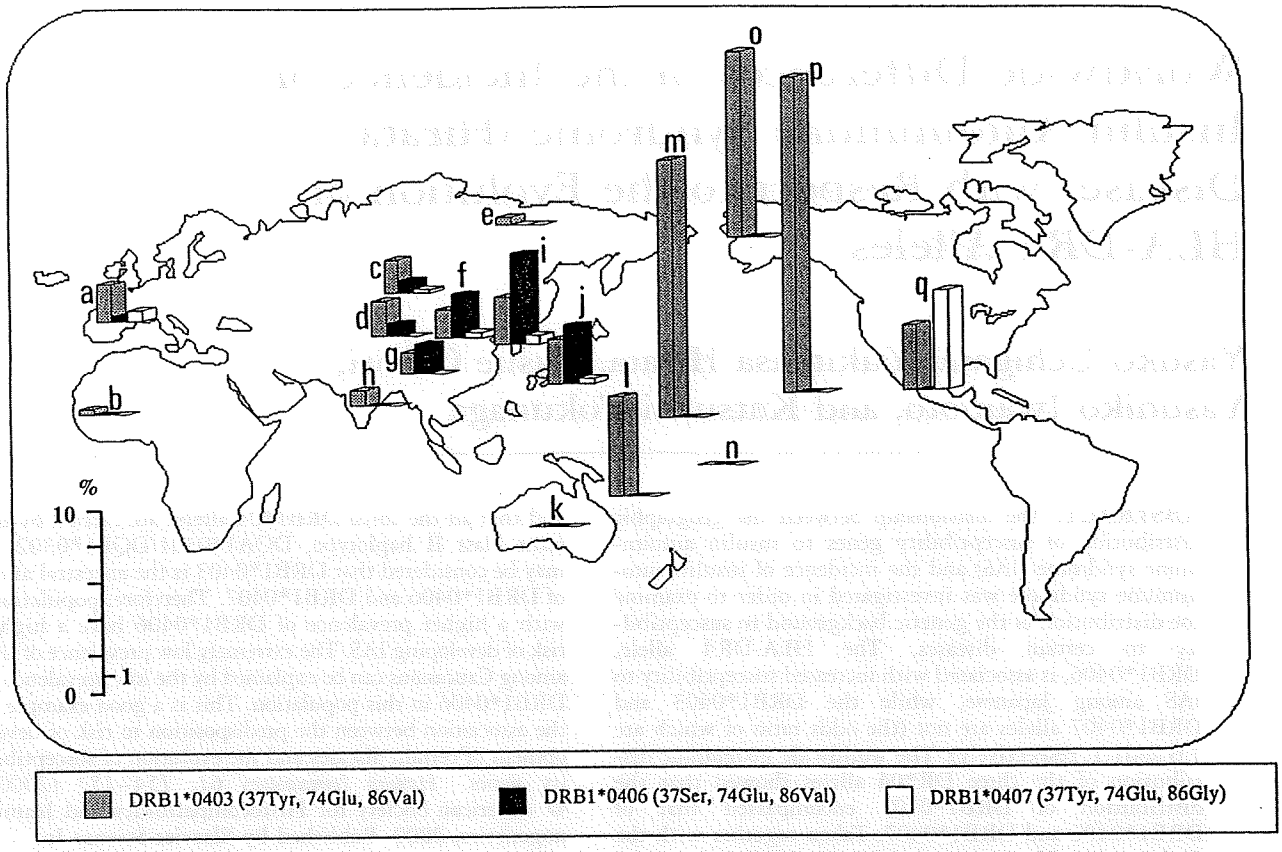

FIGURE 1 The prevalence of DRB1*0403, DRB1*0406 and DRB1*0407 alleles in representative ethnic populations. The prevalence of the three DRB1*04 alleles are shown in French (a), Senegalese (in West Africa) (b), Mongolian (c), Buyi (in South Central China) (d), Buyat (in Siberia) (e), Man Chinese (f), northern Han Chinese (g), Asian Indian (h), Korean (i), Japanese (j), Australian aborigine (k), coastal Melanesian (l), Micronesian (m), Southern Papua New Guinean (n), Tlingit (a North American Indian population in Alaska) (o), Polynesian (p), and Zuni (in New Mexico, USA), (q) populations.

We observed a strong association between IAS (Hirata disease) and the possession of the human leukocyte antigen (HLA)-DR4 in Japanese (48 out of 50 subjects vs. control, odds ratio 39.6) [10, 11]. The Japanese IAS patients possessed only three DR4 alleles: HLA-DRB1*0403, DRB1*0406, and DRB1*0407. In the present study, the geographical distribution of these three DRB1*04 alleles was investigated in order to understand their relationship to the incidence of IAS around the world.

MATERIALS AND METHODS

The prevalence of DRB1*0403, DRB1*0406, and DRB1*0407 alleles among Japanese [12] were compared with those in Senegalese (in West Africa), French, Buyi (in South Central China), Tlingit (in Alaska), Asian Indian, and Zuni (in New Mexico) populations reported in our study [12], in Australian Aborigine population reported by Gao et al. [13], in Southern Papua New Guinean population from our study [14], in coastal Melanesian population reported by Gao et al. [15], in Polynesian and Micronesian populations reported by Gao et al. [16], in northern Han Chinese, Man Chinese, Korean and Buyat (in Siberia) populations reported in our study [17], in Mongolian population reported in our study [18].

The aforementioned studies used PCR-MPH (microtiter plate hybridization) [19, 20], PCR-RFLP (restriction fragment length polymorphism) [12], and PCR-SSCP (single-strand conformation polymorphism) [21].The aforementioned studies used PCR-MPH microtiter plate hybridization) [19, 20], PCR-RFLP (restriction fragment length polymorphism) [12], and PCR-SSCP (single-strand conformation polymorphism) [21].The aforementioned studies used PCR-MPH (microtiter plate hybridization) [19, 20], PCR-RFLP (restriction fragment length polymorphism) [12], and PCR-SSCP (single-strand conformation polymorphism) [21].

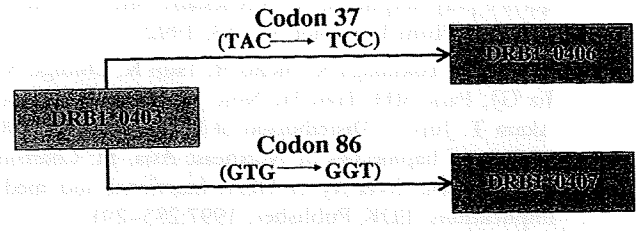

FIGURE 2 Hypothesis of evolutionary relationship among DRB1*0403, DRB1*0406, and DRB1*0407 alleles.

RESULTS

Figure 1 shows the geographical distribution of the prevalence of the three DRB1*04 alleles in aforementioned representative ethnic populations, based on the observations of other groups [13–18] as well as our own [12, 17]. Among the three, the prevalence of DRB1*0403 was found to have wide distribution across the various ethnic populations. A relatively higher prevalence of DRB1*0403 was observed in the Tlingit (North American Indians in Alaska) (11.3%), Polynesian (19.1%), and Micronesian (15.5%) populations. The prevalence of DRB1*0406 was the highest in East Asian populations, in the order of Mongolian (0.7%) and Northern Han Chinese (1.8%) populations, Man Chinese (2.6%), Korean (5.4%), and Japanese (3.5%) populations. However, the prevalence of DRB1*0407 was a low throughout Eurasia, but high in the Zuni population of New Mexico (6.0%) in North America.

DISCUSSION

DRB1*0403, DRB1*0406, and DRB1*0407 alleles share Glu at position 74 in the DRB1 molecule. There are only a few differences between the DRB1 molecule-nucleotide sequences of DRB1*0403 and those of DRB1*0406 and DRB1*0407: codon 37 is TAC (Tyr) in DRB1*0403 and DRB1*0407 while it is TCC (Ser) in DRB1*0406, and codon 86 is GTG(Val) in DRB1*0403 and DRB1*0406 while it is GGT(Gly) in DRB1*0407. However, all these DR4 alleles are carried by the same class II haplotype, DQA1*0301/DQB1*0302 [12, 21]. In this study, the geographic distribution of the three DRB1*04 alleles showed that the distribution of DRB1*0403 encompassed that of both DRB1*0406 and DRB1*0407. Therefore, DRB1*0403 can be considered as being the ancestral allele of DRB1*0406, and DRB1*0407. DRB1*0406 was possibly generated from the ancestral allele, that is DRB1*0403, independently by a single nucleotide substitution at codon 37 (Fig. 2), and DRB1*0407, independently, from the same ancestral allele (DRB1*0403) by two nucleotide substitutions or by gene conversion with a short segment at codon 86 (Fig. 2).

Predisposition to IAS among Japanese was strongly associated with DRB1*0406 (42 out of 48 DR4-positive patients, odds ratio 56.6) [22]. The odds ratio for DRB1*0403 was 1.6 (5 out of 48 DR4-positive patients), and that for DRB1*0407 was 1.1 (1 out of 48 DR4-positive patients). Such an association indicates that Glu at position 74 in the DRB1 molecule (shared by all the three alleles) is essential for the development of IAS, and Ser at position 37 (unique to DRB1*0406) greatly increases the predisposition to the disease [22]. This is confirmed by the finding that all patients with Graves' disease who developed IAS possessed DRB1*0406, while Graves' disease patients without IAS did not possess DRB1*0406 (odds ratio 2727) [22]. Therefore, populations with a higher prevalence of DRB1*0406 have a higher risk of developing IAS compared to populations with a lower prevalence. To date, 10 IAS patients have been identified in East Asia outside of Japan [8, 9]. All of these patients exhibited polyclonal IAAs and possessed DRB1*0406/DQA1*0301/DQB1*0302, similar to the Japanese IAS patients with polyclonal IAAs. The extremely low prevalence of IAS among caucasians can be explained by the low prevalence of DRB1*0406 among caucasians as found in this study. This is a good example of the association between the predisposition to risk of development of certain diseases and the evolution of susceptibility genes.

REFERENCES

1. Tokunaga K, Imanishi T, Takahashi K, Juji T: On the origin and dispersal of East Asian populations as viewed from HLA haplotypes. In Akazawa T, Szatmary EJE (eds.): Prehistoric mongoloid dispersals. New York, Oxford University Press, 1996:187–197.
2. Nei M, Roychoudhury AK: Evolutionary relationships of human populations on a global scale. Mol Biol Evol 10:927, 1993.
3. Neel JV, Biggar RJ, Sukernik RI: Virologic and genetic studies relate Amerind origins to the indigenous people of the Mongolia/Manchuria/southeastern Siberia region. Proc Natl Acad Sci USA 91:10737, 1994.
4. Schlosstein L, Terasaki PI, Bluestone R, Pearson CM: High association of and HLA antigen B27 with ankylosing spondylitis. N Engl Med 288:704, 1973.
5. Park MS, Terasaki PI, Ahmed AR, Tiwari JL: HLA-DRw4 in 91% of Jewish pemphigus vulgaris patients. Lancet 2:441, 1979.
6. Juji T, Satake M, Honda Y, Doi Y: HLA antigens in Japanese patients with narcolepsy. Tissue Antigens 24: 316, 1984.
7. Prochazka EJ, Terasaki PI, Park MS, Goldstein LI, Busuttil RW: Association of primary sclerosing cholangitis with HLA-DRw52a. N Engl J Med 322:1842, 1990.

8. Uchigata Y, Hirata Y: Insulin autoimmune syndrome (IAS, Hirata disease). In: Eisenbarth GS (ed.): Molecular mechanisms of endocrine and organ specific autoimmnity. Landes Bioscience (USA). 1998:133–148.
9. Uchigata Y, Hirata Y: The Insulin Autoimmune Syndrome (IAS, Hirata's Disease). Annales de Medecine Interne 15:245, 1999.
10. Uchigata Y, Kuwata S, Tokunaga K, Eguchi Y, Takayama-Hasumi S, Miyamoto M, Omori Y, Juji T, Hirata Y: Strong association of insulin autoimmune syndrome with HLA-DR4. Lancet 339:393, 1992.
11. Uchigata Y, Omori Y, Nieda M, Kuwata S, Tokunaga K, Juji T: HLA-DR4 genotype and insulin-processing in insulin autoimmune syndrome. Lancet 340:1467, 1992.
12. Imanishi T, Akaza T, Kimura A, Tokunaga K, Gojobori T: Allele and haplotype frequencies for HLA and complement loci in various ethnic groups. HLA 1991 Vol. 1. In Tsuji K, Aizawa M, Sasazuki T (eds.): New York, Oxford University Press, 1992:1065–1220.
13. Gao X, Veale A, Serjeantson SW: HLA class II diversity in Australian Aborigines: unusual HLA-DRB1 alleles. Immunogenetics 36:333, 1992.
14. Yoshida M, Ohtsuka R, Nakazawa M, Juji T, Tokunaga K: HLA-DRB1 frequencies of non-Austronesian-speaking Gidra in South New Guinea and their genetic affinities with Oceanian population. Am J Phys Anthrop 96:177, 1995.
15. Gao X, Bhatia K, Trent RJ, Serjeantson SW: HLA-DR, DQ nucleotide sequence polymorphisms in five Melanesian populations. Tissue Antigens 40:31, 1992.
16. Gao X, Zimmet P, Serjeantson SW: HLA-DR, DQ sequence polymorphisms in Polynesians, Micronesians, and Japanese. Hum Immunol 34:153, 1992.
17. Tanaka H, Tokunaga K, Inoko H, Tsuji K, Chimge N-O, Jia GJ, Park MH, Han H, Nose Y, Nagao N, Saitou S, Akaza T, Juji T: Distribution of HLA-A, B, and DRB1 alleles and haplotypes in Northeast Asia. In: Charron D (ed): Genetic diversity of HLA: functional and medical implications. EDK Publisher, 1997:285–291.
18. Chimge N-O, Tanaka H, Kashiwase K, Ayush D, Tokunaga K, Saji H, Akaza T, Batsuuri J, Juji T: The HLA system in the Mongolian population. Tissue Antigens 49:477, 1997.
19. Kawai S, Maekawajiri S, Tokunaga K, Juji T, Yamane A: A simple method of HLA-DRB typing using enzymatically amplified DNA immobilized probes on microtiter plate. Hum Immunol 41:121, 1994.
20. Kawai S, Maekawajiri S, Tokunaga K, Kashiwase K, Miyamoto M, Akaza T, Juji T, Yamane A: Routine low and high resolution typing of HLA-DRB gene using PCR-MPH (Microtiter Plate Hybridization) method. Eur J Immunogenet 23:471, 1996.
21. Bannai M, Tokunaga K, Lin L Kuwata S, Mazda T, Amaki I, Fujisawa K, Juji T: Discrimination of human HLA-DRB1 alleles by PCR-SSCP (single strand conformation polymorphism) method. Eur J Immunogenet 21:1, 1994.
22. Uchigata Y, Tokunaga K, Nepom G, Bannai M, Kuwata S, Dozio N, Benson EA, Ronningen KS, Spinas GA, Tadokoro K, Hirata Y, Juji T, Omori Y: Differntial immunogenetic determinants of polyclonal insulin autoimmune syndrome (Hirata's disease) and monoclonal Insulin autoimmune syndrome. Diabetes 44:1227, 1995.

Methimazole 開始4年後に診断されたインスリン自己免疫症候群の1例

稲葉　利敬[*1]　六角久美子[*1]　板橋　直樹[*1]　井手野順一[*1]
長坂昌一郎[*1]　本多　一文[*1]　岡田　耕治[*1]　横山　水映[*2]
石川　三衛[*3]　齊藤　寿一[*4]　内潟　安子[*5]　岩本　安彦[*5]
石橋　　俊[*1]

要約：症例は69歳，女性．1993年(平成5年)10月Graves病と診断されmethimazole(MMI)投与を開始．1997年(平成9年)7月より夜間空腹感を自覚し，低血糖症を指摘された．インスリン注射歴のないこと，血中インスリンの異常高値，高力価のインスリン抗体の存在からインスリン自己免疫症候群と診断．同年7月，甲状腺機能低下を認めMMIは中止されたが，低血糖症状が持続し2000年(平成12年)6月入院．75 g OGTTは糖尿病型，入院食摂取下の血糖日内変動では低血糖を認めず，絶食試験で19時間後に低血糖を認めた．HLA-DRB 1*0406を認めた．Scatchard解析の結果，本症例のインスリン抗体はポリクローナルで，そのhigh-affinity siteのK_1は$2.35\times10^{8}M^{-1}$，b_1は$1.32\times10^{-8}M$と計算された．本症例の血清は，加えたヒトインスリンに対しての本症候群に特徴的とされる低親和性，高結合能に比して高親和性でかつ低結合能を示した．本症候群では，誘因となる薬物の開始後早期に発症し，薬物中止後数カ月以内に低血糖発作が自然緩解する例が多いとされるが，本例は，MMI開始4年後に診断され中止後も症状が持続する，特異な臨床経過を示した症例であった．

Key words：①methimazole　②インスリン自己免疫症候群　③低血糖　④HLA
〔糖尿病46(10)：787～790, 2003〕

はじめに

インスリン自己免疫症候群は，自発性低血糖，インスリン注射歴がないこと，血中インスリンの異常高値，インスリン抗体陽性を満たす疾患であり，1970年に平田ら[1]により報告されて以来，現在までに200余例の報告がある．発症誘因としてSH基を持つ薬物投与が関与していると考えられる症例が多いが，特発的な発症もみられる[2,3]．通常SH基を有する薬物の開始から約2～6週の間に最初の低血糖発作が出現し[3]，低血糖発作の持続期間は1カ月以内が34%，1カ月～1年が60%，1年以上が6%であり[3]，80%以上の症例は，薬物中止後数カ月以内に低血糖発作が自然緩解すると報告されている[4,5]．今回我々は，methimazole(MMI)投与開始4年後にインスリン自己免疫症候群と診断され，MMI中止後も3年にわたり症状が持続した症例を経験したので，インスリン抗体の解析を含めて報告する．

症　例

患者：69歳，女性．

主訴：夜間空腹感，眼のちらつき．

既往歴：69歳で高血圧を指摘されbenidipine 4 mg/

[*1]自治医科大学内科学講座内分泌代謝学部門(〒329-0498　栃木県河内郡南河内町薬師寺3311-1)
[*2]済生会宇都宮病院内科(〒321-0974　栃木県宇都宮市竹林町911-1)
[*3]自治医科大学大宮医療センター総合医学1(〒330-8503　埼玉県大宮市天沼町1-847)
[*4]社会保険総合中央病院(〒169-0073　東京都新宿区百人町3-22-1)
[*5]東京女子医科大学糖尿病センター(〒162-8666　東京都新宿区河田町8-1)
連絡先：稲葉利敬(〒329-0498　栃木県河内郡南河内町薬師寺3311-1　自治医科大学内科学講座内分泌代謝学部門)

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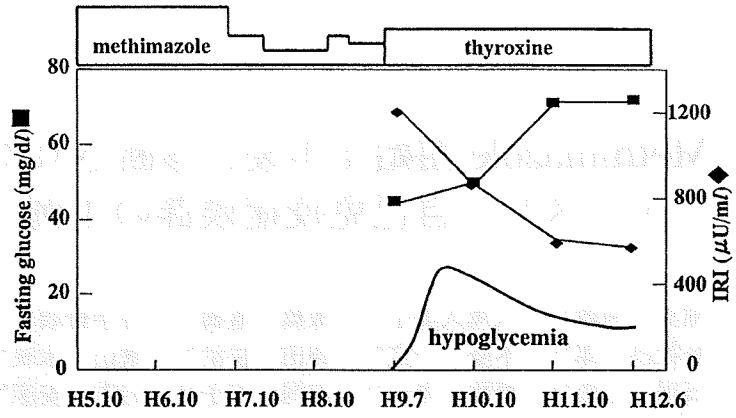

Fig. 1　Clinical course of the patient

Table 1　Endocrinological findings

ACTH	17 pg/ml	cortisol	12.0 μg/dl		
TSH	0.65 μU/ml	free T_3	1.98 pg/ml	free T_4	1.20 ng/dl
TRAb	57.6%	TSAb	311%	TSBAb	49.0%
TGHA	1638400	MCHA	4096000		
IRI	568 μU/ml	CPR	7.55 ng/ml		
Anti-insulin antibody		NSB 91% (free IRI 11 μU/ml, total IRI 1,100 μU/ml)			
Anti-GAD antibody	<4 U/ml				
ICA	(−)				
HLA-DR 4/8	(DRB 1*0406/0803)				

日を内服中．インスリン注射歴はなし．

家族歴：長女がGraves病．

現病歴：1993年(平成5年)10月(62歳)，発汗過多を自覚し，近医を受診．甲状腺ホルモン，抗TSHレセプター抗体高値よりGraves病と診断され，MMI 30 mg/日の投与が開始された．同年12月より甲状腺ホルモン値は低下しMMIは漸減された．1997年(平成9年)7月より，夜間空腹感，眼のちらつきを自覚した．インスリンの注射歴なく，空腹時血糖48 mg/dl，血中インスリン値1,000 μU/ml，インスリン抗体陽性(結合率79%)からインスリン自己免疫症候群と診断された．同時期に甲状腺機能低下を認めMMI投与は中止，サイロキシン補充が開始された．その後も低血糖症状が持続するため，2000年(平成12年)6月，精査目的で当院入院となった(Fig. 1)．

入院時現症：身長143.0 cm，体重49.0 kg，血圧150/90 mmHg，脈拍78/分・整，体温35.8℃，意識清明，眼瞼結膜に貧血なし，眼球結膜に黄疸なし，頸部に七条分類2度，びまん性で弾性硬の甲状腺腫あり，胸腹部に異常なし，神経学的所見に異常なし．

入院時検査所見：血液学的検査所見，一般生化学的検査所見に異常なし．空腹時血糖73 mg/dl，HbA1c 5.6%と正常範囲であった．内分泌学的検査(Table 1)では，ACTH，コルチゾールの基礎値は正常，甲状腺ホルモンはサイロキシン投与下で正常範囲であった．Thyroid stimulating hormone receptor antibody (TRAb)，thyroid stimulating antibody (TSAb)，thyroid-stimulation blocking antibody (TSBAb)，antithyroglobulin hemagglutination antibody (TGHA)，antithyroid microsomal hemagglutination antibody (MCHA)はいずれも高値であった．血中immunoreactive insulin (IRI) (ELISA法)，C peptide (CPR) (RIA法)は異常高値，インスリンの抗体への結合率は91%と高値であった．HLA-DRは4/8(DRB 1*0406/0803)であり，DRB 1*0406を認めた．なおHLA-A，B，Cについては検索し得なかった．

75 gOGTTでは，血糖の前値73 mg/dl，120分値230 mg/dlであり，糖尿病型を示した．入院食摂取下の血糖日内変動では，一日を通して低血糖発作は認めなかったが，絶食試験では血糖値は徐々に低下し，約19時間後に発汗過多，眼前暗黒感が出現し，低血糖を認めた(Table 2)．

Table 2 Diurnal variation of plasma glucose

	fed wih standard meals		fasted	
	plasma glucose		plasma glucose	IRI(ELISA)
8:00	76	←breakfast	74	559
10:00	130		78	541
12:00	81	←lunch	78	497
14:00	120		76	421
18:00	99	←dinner	65	380
20:00	164		64	326
0:00	84		57	294
3:00	86		45	278
6:00	83		43	263
	(mg/dl)		(mg/dl)	(μU/ml)

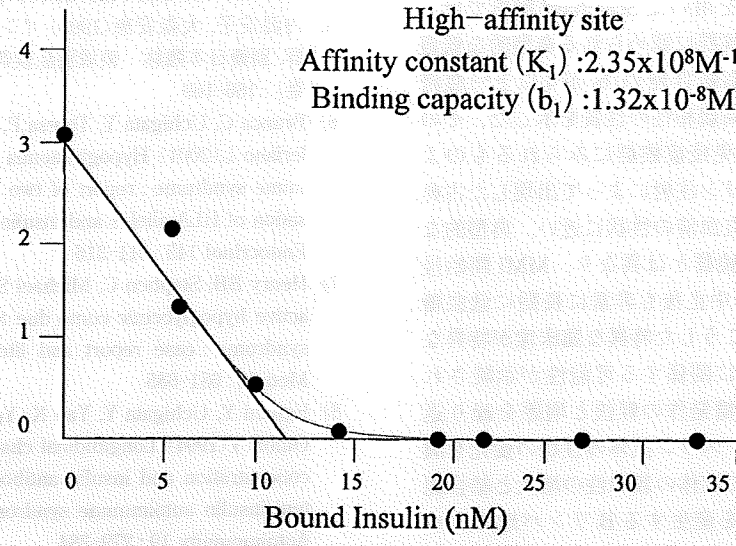

Fig. 2 Scatchard analysis
Scatchard analysis of insulin autoantibody in the patient, using ^{125}I-human insulin. Serum, with insulin removed by dextran-coated charcoal, was used for the Scatchard analysis[6].

インスリン抗体は，specific precipication 法による免疫グロブリンクラスの検討ではIgG抗体，全血清を用いたScatchard解析[6]はポリクローナル抗体に特徴的なものであり，high-affinity site の K_1 および b_1 は，$K_1=2.35\times10^8\ M^{-1}$，$b_1=1.32\times10^{-8}$ M であった(Fig. 2)．また，甲状腺エコーは慢性甲状腺炎に一致する所見であった．

入院後経過(Fig. 1)：本症例では，MMI開始約4年後の1997年(平成9年)7月より低血糖発作が認められたが，経過と共に空腹時血糖値は上昇し，低血糖発作の頻度は低下した．今回入院中，入院食摂取下では低血糖発作はなく，絶食下で初めて低血糖が出現した(Table 2)．入院中の検査結果より，食事時間を規則正しくし，低血糖出現時は補食で対応するよう指導した．退院後約2年が経過したが，現在でも月に1回程の早朝空腹時の低血糖発作が持続しており，インスリン抗体の高値(結合率：平成13年5月75%，7月80%，平成14年5月92%)も持続しており，ステロイドパルス療法等も検討中である．

考　察

本症例は自発性低血糖を認め，インスリン注射歴のないこと，血中インスリン異常高値，高力価インスリン抗体の存在からインスリン自己免疫症候群と診断された．1993年末までの各国からの報告例では，全報告例は244例，そのうち日本人での報告が212例(86.4%)と大半を占める[3]．海外からの報告は東洋人に多く，その他米国黒人の症例などが散見されるが，欧米

人には少ない[6,7]．インスリン自己免疫症候群は，誘因としてSH基を持つ薬物の関与が指摘されている[2]．日本人の報告例212例中44例にMMI，38例にtiopronin，7例にglutathione内服歴が認められている[3]．また誘因となる薬物の開始2～6週後と早期に発症し，薬物中止後数カ月以内に低血糖発作が自然緩解する例が多いと報告されている[3]．本症例の場合も誘因としてMMIが最も考えられる．しかし他の症例と異なり，MMI開始から発症までの期間が長く，MMI中止後も低血糖の経過が長いことが特徴的であった．このような症例の報告はほとんどなく，その機序は不明である．ただし，本症例のインスリン自己免疫症候群の発症にMMIが関与せず，特発的に発症した可能性も完全には否定できない．

今回の入院時の血清を用いたScatchard解析では，インスリン自己免疫症候群に認められる典型的な低親和性（K_1が1未満[8]）で高結合能の（b_1が4以上[8]）血清性状と比較して，より高親和性の性質を示した．その性状はインスリン自己免疫症候群にみられるものより，むしろ動物インスリン注射によって出現した古典的なインスリン抗体含有血清の性状に近い．典型的なインスリン自己免疫症候群とは異なり，MMI開始後早期に発症せず，MMI中止後も非常に長期に低血糖発作が持続しており，こうした特異な臨床像が特異な性状のインスリン抗体に関係する可能性が示唆された．Eguchiらは，低血糖発作の軽快と増悪を繰り返した症例で経時的にインスリン抗体の性状の変化を調べ，発作の軽快に伴って抗体の親和性の増大と結合能の低下を観察し，抗体を産生するBリンパ球のクローン変化を想定している[8]．本症例では，低血糖発作が頻回に認められていた時期の血清の解析は施行し得なかったが，このような時期の血清の外来性ヒトインスリンに対する結合の性状は今回のものより低親和性，高結合能であった可能性が高い．入院時には親和性の低下と血中濃度の低下が本インスリン抗体にすでに起こっていたのかもしれない．

インスリン自己免疫症候群の発症にはHLA遺伝子の関与が考えられている[9~11]．本症候群ではHLA-DRB 1*0406を有するものが84％と高率であると報告されており[12]，本症例もHLA-DRB 1*0406を認めた．

Graves病の治療としてMMIを使用する症例は多い．MMIの副作用としてインスリン自己免疫症候群の頻度は高くはないと思われるが，MMI投与中は，投与早期のみならず，投与期間が長期化した後でも常に本症候群の発症の可能性を念頭におく必要があると思われた．

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文　献

1) 平田幸正，石津　汪，大内伸夫，本村正治，安部宗顕，原泰寛，若杉英之，高橋　功，坂野弘幸，田中正人，河野弘道，兼崎　勉（1970）インスリン自己免疫を示した自発性低血糖症の1例．糖尿病13：312-320
2) 平田幸正，内潟安子（1997）インスリン自己免疫症候群．日本臨牀55　増刊　糖尿病（2）：786-791
3) 平田幸正（1994）インスリン自己免疫症候群．日内会誌83：77-84
4) Uchigata Y, Eguchi Y, Takayama-Hasumi S, Omori Y（1994）Insulin autoimmune syndrome（Hirata disease）：clinical features and epidemiology in Japan. Diabetes Res Clin Pract 22：89-94
5) 内潟安子，大森安恵（1993）インスリン自己免疫症候群．別冊日本臨牀　領域別症候群2　内分泌症候群（下巻）：166-169
6) Branca C, Uchigata Y, Teresa P, Maria AS, Luis S, Valeriano L（2001）Hypoglycaemia due to insulin autoimmune syndrome：report of two cases with characterisation of HLA alleles and insulin autoantibodies. Eur J Endocrinol 145：311-316
7) Henry BB, Stephen C, Michael SS, Frank L（1992）Reactive hypoglycemic coma due to insulin autoimmune syndrome：case report and literature review. Am J Med 92：681-685
8) Eguchi Y, Uchigata Y, Yao K, Yokoyama H, Hirata Y, Omori Y（1994）Longitudinal changes of serum insulin concentration and insulin antibody features in persistent insulin autoimmune syndrome（Hirata's disease）. Autoimmunity 19：279-284
9) Uchigata Y, Kuwata S, Tokunaga K, Eguchi Y, Takayama-Hasumi S, Miyamoto M, Omori Y, Juji T, Hirata Y（1992）Strong association of insulin autoimmune syndrome with HLA-DR 4. Lancet 339：393-394
10) Uchigata Y, Omori Y, Nieda M, Kuwata S, Tokunaga K, Juji T（1992）HLA-DR 4 genotype and insulin-processing in insulin autoimmune syndrome. Lancet 340：1467
11) Uchigata Y, Kuwata S, Tsushima T, Tokunaga K, Miyamoto M, Tsuchikawa K, Hirata Y, Juji T, Omori Y（1993）Patients with Graves' disease who developed insulin autoimmune syndrome（Hirata diseasee）possess HLA-Bw 62/DR 4 carrying DRB 1*0406. J Clin Endocrinol Metab 77：249-254
12) Uchigata Y, Tokunaga K, Garald N, Bannai M, Kuwata S, Nicoletta D, Edward AB, Kjersti SR, Glatgen AS, Tadokoro K, Hirata Y, Juji T, Omori Y（1995）Differential immunogenetic determinants of polyclonal insulin autoimmune syndrome（Hirata's disease）and monoclonal insulin autoimmune syndrome. Diabetes 44：1227-1232

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LONGITUDINAL CHANGES OF SERUM INSULIN CONCENTRATION AND INSULIN ANTIBODY FEATURES IN PERSISTENT INSULIN AUTOIMMUNE SYNDROME (HIRATA'S DISEASE)

YOKO EGUCHI, YASUKO UCHIGATA, KENSHI YAO*, HIROKI YOKOYAMA, YUKIMASA HIRATA AND YASUE OMORI

*Diabetes Center, Tokyo Women's Medical College, *Fukuoka University Chikushi Hospital*

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In a 56-year-old woman with granulomas of gold thioglucose in her hips, who developed insulin autoimmune syndrome, the relationships among the frequency or severity of hypoglycemic attacks, serum insulin (IRI) concentration, and characteristics of insulin antibodies were investigated during the clinical course with steroid treatment and two resection operations for the gold-thioglucose granulomas. When hypoglycemia was severe, the total IRI level was elevated, and Scatchard analysis showed that a high-affinity (k1), low-capacity (b1) population of antibodies had a relatively low affinity constant and very high binding capacity compared with the same population of antibodies in insulin-treated diabetic patients. When the attacks were relieved by steroid treatment and/or granuloma resection operation, the total IRI level was decreased and the high-affinity (k1), low-capacity (b1) population of antibodies showed a higher affinity constant and a lower binding capacity than those during the attacks. This indicated that the antibodies changed their characteristics to release insulin into the serum. The k1/b1 population of insulin antibodies with the lower affinity constant and higher binding capacity may easily release human insulin into the serum, leading to hypoglycemia. The longitudinal change of the k1/b1 population suggests a clonal change of the B cells producing the insulin antibody in insulin autoimmune syndrome.

KEY WORDS: Insulin antibody, Scatchard analysis, hypoglycemic attack.

INTRODUCTION

Insulin autoimmune syndrome (IAS), which was first reported by Hirata *et al.*[1] in 1970, is the third leading cause of severe postprandial hypoglycemia[2] and is characterized by the following diagnostic criteria: spontaneous hypoglycemia without evidence of exogenous insulin administration, a high level of total serum immunoreactive insulin, and the presence of a high titer of insulin antibody. Forty-three percent of IAS patients had taken medication such as methimazole, α-mercaptopropionyl glycine, or glutathione — all sulfhydryl compounds- prior to the attacks[3,4]. Recently, we revealed another interesting finding of a strong association of IAS with HLA-DRB1*0406/DQA1*0301/DQB1*0302[5].

Recurrent hypoglycemic episodes usually subside within one year, along with a decrease in the titer of insulin autoantibodies and serum immunoreactive insulin[6]. However, it remains to be determined how insulin autoantibodies develop in patients and how hypoglycemia subsides spontaneously. We reported on a 56-year-old woman with granulomas of gold thioglucose, who exhibited recurrent bouts of hypoglycemic attacks for more than 5 years[7]. Her hypoglycemic attacks almost completely ceased after resections of the hip granulomas after the 6th year. In this study, her unusual and persistent hypoglycemia due to insulin autoimmune syndrome was evaluated from serum total and free insulin concentrations, and the features of insulin antibody were analyzed preiodically by Scatchard analysis and used to classify the antibodies into a high-affinity, low-capacity population and a low-affinity, high-capacity population.

MATERIALS AND METHODS

Patient

The profile of the 56-year-old patient with insulin autoimmune syndrome has been previously reported[7]. She had her first hypoglycemic attack two years after

Correspondence to: Dr Yasuko Uchigata, Diabetes Center, Tokyo Women's Medical College, 8–1 Kawada-cho, Shinjuku-ku, Tokyo 162, Japan.