A Case of Insulin Autoimmune Syndrome Associated with* Small Insulinomas and Rheumatoid Arthritis

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Abstract

Twenty five cases of insulin autoimmune syndrome including this case have been reported so far without having the pathogenesis clarified. This paper describes a case which suggests one aspect of pathogenesis. The patient, a housewife concurrently had insulinoma and severe rheumatoid arthritis, complaining of hypoglycemic syncope attacks. During the attacks her blood sugar levels ranged from 19 to 22 mg%. Her serum extractable immunoreactive insulin (IRI) and insulin binding antibody levels were 557 µU/ml and 0.390 mU/ml, respectively. γ-Globulin-bound insulin was also measured electrophoretically. Bio-Gel P 10 column chromatography eluted almost all IRI at the void volume at pH 7.4 and a smaller but significant IRI peak also at pH 3.0. Selective angiography revealed a tumor-like staining in the pancreas body. Pancreatectomy relieved her of hypoglycemic attacks. Histology disclosed two small insulinomas.

Insulinoma, rheumatoid arthritis and insulin autoimmune syndrome coexisted in this case, suggesting some causal relationship among them.

To the best of our knowledge, about twenty five cases of insulin autoimmune syndrome including our case (Fushimi et al., 1980) have been reported since the first description by Hirata et al. (1974). Its pathogenesis has been far from being clarified, however. Kuzuya et al. reported a case of insulinoma associated with the production of insulin antibody, although the binding titer was low and hypoglycemia was thought to be unrelated to insulin autoimmunity in their case. Among roughly 1,000 cases of insulinoma reported hitherto, however, there has been no other case of proven insulin autoimmunity.

On the other hand, histologic studies of the pancreas of four patients with typical insulin autoimmune syndrome revealed a nearly normal pancreas or hyperplasia of the islets but no insulinoma (Hirata et al., 1975; Ohneda et al., 1974; Sato et al., 1973). Recently we encountered a case of insulin autoimmune syndrome. The patient was concurrently suffering from severe rheumatoid arthritis, and in addition, had small insulinomas. The study of this case has brought provided intriguing results that may throw some light on the disease from the pathogenetic angle.

Case Reports and Methods

In 1971, a 56-year-old Japanese housewife visited the outpatient clinic of our
orthopedic department and was diagnosed as having rheumatoid arthritis. Since then the patient has been given occasional intraarticular injections of corticosteroid. The other drugs used were some antacids and digestive enzyme preparations. In January, 1977, she was hospitalized on account of intensified pain in all joints. At the end of the month she had a syncope attack before breakfast with a blood sugar level of 19 mg\% and syncope abated completely after intravenous glucose administration, and during the following month she experienced five episodes with blood sugar levels ranging from 19 to 22 mg\% before breakfast or postprandially.

The family history showed nothing remarkable.

Gastrectomy for a gastric ulcer was done in 1972 and appendectomy in 1973.

**Physical Findings**

Height: 153 cm. Body weight: 41.5 kg. Face: round-faced slightly. Blood pressure: 118/74 mmHg. Skin and palpebral conjunctiva: slightly anemic. No lymphadenopathy was found. Fingers and toes showed marked swan neck deformities. Knee, ankle and shoulder joints were swollen. All joints showed impaired movements.

**Routine laboratory findings**

Blood analysis showed RBC $364 \times 10^4$ mm$^3$, Hb 9.3 g/100 ml, WBC 6900/mm$^3$ (band 0\%). Urinalysis was negative. Platelet count was $34 \times 10^4$/mm$^3$. Erythrocyte sedimentation rate was 76 mm/hour. RA test was positive. CRP was trace-positive. Thyroid test, microsome antibody, DNA test and ANF test were negative. Serum total protein was 6.6 g/100 ml (albumin 51.0\%, $\alpha_1$-globulin 4.6\%, $\alpha_2$-globulin 10.2\%, $\beta$-globulin 11.3\%, $\gamma$-globulin 22.6\%). Cholesterol, Na, K, Cl and Ca levels were normal. IgM and IgG elevated slightly, to 266 mg/100 ml and 1900 mg/100 ml respectively. The IgA level was normal. Liver function tests, and urea N and creatinine concentrations were normal. The carcinoembryonic antigen concentration and $\alpha$-fetoprotein level were not increased. Serum Fe level was 27 $\mu$g/100 ml. Irosorb was 300 $\mu$g/100 ml. EEG and EEG were within normal limits. The chest x-ray was almost negative. Bone x-ray examination unmasked osteoporotic and rheumatic changes.

**Endocrinologic findings**

Thyroid function was normal. HGH levels were also normal. Adrenal function appeared subnormal (cortisol levels were 1.2-2.0 $\mu$g/100 ml in the morning, urine 17KS ranged from 1.7 to 2.7 mg/day, 17 OHCS from 1.7 to 2.7 mg/day, 17OHCS from 0.4 to 1.5 mg/day), but a rapid ACTH test showed normal response. Serum IRI and C-peptide were determined by a routine double antibody method using Dai-ichi C-peptide kit. Fasting IRI levels were 120-900$\mu$U/ml. Fasting C-peptide levels were 1.0-14.0$\mu$g/ml. Gastrin levels were within the normal range (40-70 pg/100 ml). The OGTT are shown in Fig. 1. The blood glucose curve was diabetic, with high levels of IRI and CPR. Glucagon was determined by Dr. Tatsuo Matsuyama, Second Dept. of Medicine, Osaka University School of Medicine, using pancreatic glucagon specific antibody AGS 18. Glucagon response to arginine infusion was normal (Fig. 2). Intravenous insulin administration (regular insulin 0.10 U/kg body weight) caused hypoglycemia and a normal growth hormone response (Fig. 2).

**Clinical course**

Despite the fact that the patient had no history of insulin injection, serum levels of IRI and insulin binding antibodies were notably raised. On the basis of these data, this was diagnosed as insulin autoimmune syndrome associated with rheumatoid arthritis. However, the selective angiography
Fig. 1. 100 g Glucose tolerance test.
Serum IRI and C-peptide were estimated by double antibody method of radioimmunoassay.

Fig. 2. Arginine and insulin tolerance tests.
For arginine tolerance test 10% solution was given intravenously at a rate of 1 g/min for 30 minutes (---). For insulin tolerance test 0.1 U/kg body weight of regular insulin was given intravenously in one shot.
showed a staining (3×3 cm) in the pancreas body which was suggestive of a tumor. On suspicion of insulinoma the patient was laparotomized. Part of the organ was enlarged, though its consistency was not different from that of the remainder. The body of the pancreas, including the stained region and the tail, were resected. No tumor was found in that region, but when formaline-fixed specimens were cut into 1 cm widths, two small insulinomas were found, one of which was 1.3×1.5–2.8 mm, in the body of the pancreas distal to the tumor-like staining. Gomori’s trichrome and aldehyde-fuchsin staining were done to show that almost all tumor cells were B cells which were in a ribbon or string like arrangement typical of insulomas (Fig. 3A). Immunofluorescent studies were kindly undertaken by Prof. Tsuneo Fujita, Dept. of Anatomy, Niigata University School of Medicine. The insulinomas were not positive for insulin, C-peptide, glucagon or somatostatin, but all other normal islets were positive (Fig. 3B-C). All tissue specimens were examined and only two hypertrophic islets (0.4×0.2 mm and 0.3×0.3 mm) were detected and all the other islets were <200 μ in diameter. Insulin extraction could not be carried out, because tumors were too small. After the operation, the patient was free from hypoglycemic attacks and IRI has returned to a level of 10 to 30 μU/ml.

Studies of insulin binding antibody
1. Ethanol precipitation method (Nakamura et al., 1972)

Each patient and control serum, 0.1 ml, was incubated with 125I-insulin, 0.1 ml, of
Fig. 3. B: Insulinoma. Immunofluorescent stain using insulin antibody.

Fig. 3. C: Normal islet. Immunofluorescent stain using insulin antibody.
an appropriate count at 4°C for 24 hours, its radioactivity was measured and then 80% cold ethanol, 2 ml, was added and mixed. After centrifugation at 3000 rpm for 30 minutes at 4°C, radioactivity of the sediment was measured.

2. Dextran-charcoal method (Herbert et al., 1965)

Serum 0.1 ml, $^{125}$I-insulin 0.1 ml and 0.1 ml Veronal buffer, pH 7.4, 0.3 ml were mixed and incubated for 48 hours at 4°C. A 2.5% dextran charcoal solution, 0.5 ml, was then added. Centrifugation was performed at 3000 rpm for 30 minutes. Total and bound radioactivity were assayed.

3. Separax electrophoresis

The following three samples were prepared: 1) The first one comprising 20 µl each of patient serum, pork insulin (80 U/ml) and $^{125}$I-insulin. 2) The second comprising 20 µl each of patient serum, $^{125}$I-insulin and normal saline. 3) The last comprising 20 µl each of control serum, $^{125}$I-insulin and normal saline. These samples were incubated at 4°C for 24 hours. Each sample was submitted for separax electrophoresis. The current was adjusted to 1.5 mA/cm and phoresis was stopped at 1 hour. Then, the separax was cut into 2 mm widths and radioactivity was counted.

4. Antibody class

Serum, 20 µl, was incubated with $^{125}$I-insulin, 0.1 ml, at 4°C for 24 hours and anti-human immunoglobulin rabbit serum (anti-IgA, IgE, IgG, IgM, κ or λ), 0.2 ml, was added. The solution thus prepared was incubated at 4°C for 48 hours. Thereafter, it was subjected to measurement of total radioactivity and then was centrifuged at 3000 rpm for 10 minutes. The resultant supernatant was discarded and the sediment was washed 3 times with 1 ml of cold normal saline and then radioactivity was counted.

5. Column chromatography

Big-Gel P 10 column, 1 × 50 cm was equilibrated with 0.1 M glycine buffer, pH 3.0 or 0.1 M phosphate buffer, pH 7.4. One ml of the serum dialyzed against the same buffer was applied and eluted with the same buffer. The fraction volume was 1 ml. The IRI and C-peptide of each fraction were assayed.

6. Other studies

Total extractable IRI (Heding, 1969), C-peptide, proinsulin, insulin binding IgE, porcine and bovine proinsulin binding IgG, and A-component binding IgG were measured by Dr. L. G. Heding (Novo Co., Denmark) (Schlichtkrull et al., 1974).

Results

Serum IRI was remarkably elevated, as demonstrated in OGTT (Fig. 1) as well as at intermittent assays of a fasting level. Total extractable IRI was as high as 557 µU/ml and C-peptide (not extracted) also had high value of 3.0 ng/ml by Dr. Heding [In normal subjects C-peptide level is 1.05 ± 0.27 ng/ml (mean ± ISD) in her laboratory]. Proinsulin level was also increased (1.475 p mol/ml). Insulin binding IgG level was remarkably increased to 0.390 mU/ml (normal <0.050 mU/ml). On the other hand porcine and bovine proinsulin IgG were not detected in the serum. The A-component binding IgG was negligible (0.5 ng/ml, normal <2.5 ng/ml). The existence of a considerable amount of γ-globulin bound $^{125}$I-insulin was shown by separax electrophoresis. The bound $^{125}$I-insulin was liberated by addition of cold insulin. The percent ratio of bound to total $^{125}$I-insulin added to serum was much larger (40.0 to 65.0%) than those of control subjects (5.2 to 19.0%) (Table I) and it decreased to 29.3–38.2% postoperatively.

Bio-Gel P 10 column chromatograms of patient serum demonstrated that almost all IRI was eluted at the void volume at pH 7.4. The column chromatography carried out at pH 3.0 on three different occasions
still yielded a significant peak of IRI at the void volume (Fig. 4). C-peptide radioactivity was found in tubes 35 to 42.

The insulin binding antibody of the patient was IgG and the light chains of insulin binding globulin were of the Kappa and the Lambda type.

Discussion

Since insulin autoimmunity without insulin injections was first described by Hirata et al. (1970), only 25 cases of this autoimmunity have been reported to date including our case and the pathogenetic facet of the disease has not been unveiled yet. Our patient reported here has never received insulin injections. No family history of diabetes mellitus was found. Insulin binding antibody (IgG) and extractable serum insulin were remarkably elevated, whereas porcine and bovine proinsulin binding IgG were undetectable. A-component binding IgG to be induced by commercial insulin injection (Schlichtkrull et al., 1972: Rubenstein et al., 1969: Kawazu et al., 1975) was undetectable (shown by Dr. Heding). These data suggest that insulin binding IgG of this patient was not derived from injection of insulin, but was of an autoimmune

Table 1. Change in Ratio of Bound to Total % 

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<th>Date</th>
<th>80% Ethanol</th>
<th>2.5% Dextran</th>
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<tr>
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<tr>
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Normal range 5.2-19.0%
* Operation was performed.
nature. Some of the previously reported cases had hyperthyroidism (Hirata et al., 1974) and the other case had rheumatoid arthritis (Goldman et al., 1979). Our patient has been suffering from active rheumatoid arthritis for the development of insulin autoimmunity, though the lymphocytic infiltration as depicted by Grodsky et al. (1966) was not seen upon histologic examination of the islets. The two insulinomas of our patient were solitary and unaccompanied by hyperplasia of the other islets. These tumors appeared to be composed mainly of B cells following aldehyde and Gomori’s trichrome staining. The evidence that they were negative for insulin and C-peptide immunofluorescent staining while the normal islets were positive suggests that the tumors may have contained little insulin because characteristic B granules of pancreatic B cell, stained by aldehyde fuchsine or Gomori’s trichrome, were not insulin but some substance in the membrane sacs surrounding the B granules as suggested Fujita et al. (1968) or that insulin turnover in these insulinomas was too rapid due to a defective storage mechanism (Creuzfeldt et al., 1973).

This is a rare case of insulinoma combined with insulin autoimmune syndrome. Previously Kuzuya et al. reported an insulinoma case with a low titer of insulin binding antibody (Kuzuya et al., 1977), but typical insulin autoimmune syndrome associated with insulinoma has not been reported. It is not known whether this coexistence occurred incidentally or causally. One possibility is that insulinomas may play a role in the formation of insulin antibody by maculomolecular insulin (Yalow and Berson, 1973: Gutman et al., 1971) or abnormal insulin being secreted by insulinomas. Another possibility is that insulin autoimmunity might stimulate islets to produce an insulinoma. Insulin autoimmunity was reported to cause hyperplasia of β cells. However in this case no hypertro-

phied islets were found but other islets were quite normal in size (less than 200 μ in diameter).

Finally we would like to mention that, though no final conclusion can be drawn until much more data is studied, this presentation may supply a clue in characterizing the pathogenesis of insulin autoimmune disease.

References