T-Cell Insulitis Found in Anti-GAD65+ Diabetes With Residual β-Cell Function

A case report

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CASE HISTORY — We recently encountered a 65-year-old anti-GAD+ diabetic woman with residual β-cell function who was proved to have T-cell insulitis. The proportion of CD4+ and CD8+ cells varied among individual islets, although CD4+ cells tended to be the predominant T-cell type in the islets examined. All of the islets examined still contained insulin, suggesting that β-cell mass may have been preserved.

DISCUSSION — It is well known that lymphocytic infiltration of pancreatic islets, a condition referred to as "insulitis," is seen in acute-onset type 1 diabetes at autopsy and in biopsy specimens. However, there have been no proven cases of insulitis in type 1 diabetes with residual β-cell function. We believe that this is the first type 1 diabetic patient with residual β-cell function who was proven to have T-cell insulitis. This novel evidence will contribute to the proper classification and treatment of diabetes and to a better understanding of the pathophysiology of type 1 diabetes.

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It is well known that lymphocytic infiltration into pancreatic islets, insulitis, is seen at autopsy in acute-onset type 1 diabetic patients who have died of diabetic ketoacidosis (1–3). Insulitis has also been observed in biopsy specimens from type 1 diabetic patients in a ketotic state who have survived acute metabolic disorders (4). However, there have been no proven cases of insulitis in type 1 diabetic patients with residual β-cell function, including so-called "slowly progressive IDDM" (5) or "latent autoimmune diabetes in adults" (6). It is important to investigate whether insulitis actually exists in patients with type 1 diabetes with residual β-cell function as well as in patients in a ketotic state, because the answer will affect the proper classification (7) and treatment of diabetes. The importance of anti-GAD antibody in type 1 diabetes has recently been demonstrated (8). This antibody can be detected not only in type 1 diabetic patients in a ketotic state but also in diabetic patients with residual β-cell function (9) and is considered a good predictive marker of insulin dependence (6). Here we report proven T-cell insulitis in an older diabetic woman with residual β-cell function. Because a high titer of anti-GAD antibodies was detected in this patient, this case was considered to be type 1 diabetes with residual β-cell function. We believe that this is the first reported case of a type 1 diabetic patient with residual β-cell function who was proven to have T-cell insulitis.

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Abbreviations: FPG, fasting plasma glucose; IFN, interferon.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

CASE HISTORY — A 65-year-old Japanese woman consulted a local physician in August 1996 for weight loss that had occurred since the spring of 1996, and she was found to have diabetes (fasting plasma glucose [FPG] level, 15.4 mmol/l; HbA_{1c}, 11.1% [normal 4.3–5.8]). She was referred to our hospital with a diagnosis of type 2 diabetes in October 1996. She had no personal history of obesity (BMI >26 kg/m^2) and no family history of diabetes. Although she had no history of ketosis (no ketonuria on repeated testing), anti-GAD65 (151.0 U/ml [cutoff <1.5]; 100% sensitivity and 100% specificity of the assay in a GAD antibodies proficiency test [Immunology of Diabetes Workshop]; Lab ID number 305) and IA-2 antibodies (1.7 U/ml [cutoff <0.75] [M. Powell, S. Chen, H. Tanaka, M. Masuda, C. Beer, B. Rees Smith, J. Farmaniak, unpublished observations]) (10) were detected in the serum, and the 24-h urine C-peptide level was 9 μg/day (normal 16–120). Serum interferon (IFN)-γ was undetectable (<0.6 U/ml)(11). The patient also possessed HLA-DR4 (DRB1*0405), which is one of the major HLA types in type 1 diabetes in Japanese (other HLA types in this case were A24, B54, B35, Cw1, Cw3, DRB1*0802, DQB1*0401, and DQB1*0302 [12]). After admission to our hospital, insulin treatment was started to treat hyperglycemia, and the plasma glucose level was controlled almost to the normal range with 16 U/day of insulin. Because the patient was found to have multiple gallbladder stones, surgery was performed to remove them in November 1996. At the time of surgery, only 2 U per meal of regular insulin and 4 U of NPH insulin at bedtime (a total of 10 U/day) were used, the HbA_{1c} was 6.1%, and the FPG was 7.7 mmol/l. We think that it may have been possible to discontinue insulin at this point, but we usually use insulin when operations are scheduled, even in type 2 diabetic patients, because better glycemic control is required for safer operations; therefore, we continued treating the patient with insulin.

With institutional review board permission and written informed consent from the patient, pancreatic biopsy was performed during the operation to examine

the islets histologically (only one biopsy specimen was collected). All islets on the section were examined by a masked examiner (pathologist). Pancreatic histology revealed the existence of insulitis in the islets (Fig. 1). An immunoperoxidase study was performed on paraffin-embedded material using the avidin-biotin-peroxidase complex method. Most of the lymphocytes in the islets were T-cells (UCHL-1⁺ cells), and no B-cells (L-26⁺ cells) were detected. The proportion of CD4⁺ and CD8⁺ cells varied in individual islets, although CD4⁺ cells tended to be the predominant T-cell type in the islets examined (the ratio of CD4⁺ to CD8⁺ cells on the biopsy specimen was 9:4, 4:3, and 3:1 in three different islets counted). Small numbers of macrophages (CD68⁺) and natural killer (CD57⁺) cells were also observed. Interestingly, all of the islets examined (n = 9) still contained insulin (Fig. 2), suggesting that more β-cell mass may have been preserved than previously imagined. Actually, the proportions of β- to non–β-cells in patients and age-matched normal control subjects (n = 3) were 52 vs. 48 and 55 vs. 45%, respectively (no significant difference was observed), and no pseudoatrophic islets were observed in this case. The cellular composition of the lymphocytic infiltrate and the degree of β-cell destruction seemed to vary in individual islets, suggesting that different islets may contain different stages of disease progression.

At present, the patient's 24-h urine C-peptide level has recovered to the normal range (35 μg/day), her serum C-peptide level is 0.63 nmol/l when obtained 6 min after intravenous injection of glucagon (1 mg glucagon–stimulated serum C-peptide level), and her HbA_{1c} levels have been well maintained between 5.9 and 6.1%; however, she is still being treated with 8 U/day of insulin because the recommended treatment for type 1 diabetes with residual β-cell function is insulin.

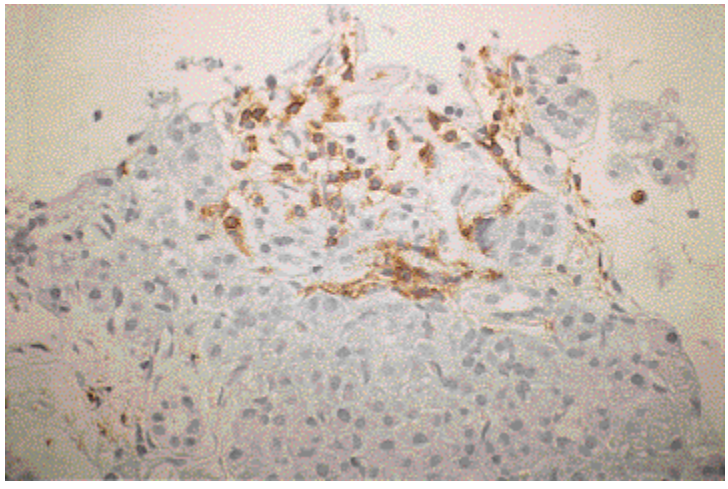

Figure 1—*Typical insulitis lesion observed in this patient. Cells positive for leukocyte common antigen exist in the islet lesion.*

DISCUSSION — In "typical" type 1 diabetes involving a ketotic state, the presence of insulitis has been proved at autopsy (1–3) and by biopsy (4), whereas there has never been a proven case of insulitis in type 1 diabetes with residual β-cell function. We provide the first report of the existence of insulitis in a patient with type 1 diabetes with residual β-cell function.

Bottazo and colleagues (13) reported finding no insulitis in anti-GAD⁺ nondiabetic polyendocrine patients. They stated that no islet autoantibodies other than β-selective GAD-specific islet cell antibodies were detected in their patients. On the other hand, our patient, who was proven to have insulitis, was positive for IA-2 (protein tyrosine phosphatase–like molecule) antibodies, which may be associated with a more aggressive disease pathogenesis than GAD antibodies (14).

In typical type 1 diabetes, autopsy or biopsy has been performed after severe destruction of pancreatic islets, and the majority of infiltrating cells have been CD8⁺ cells or macrophages (4,15). In our study, however, we obtained a pancreatic specimen in a nonketotic state, and the proportion of CD4⁺ and CD8⁺ cells was variable in individual islets, although CD4⁺ cells tended to be the predominant T-cell type in the islets examined. We may have detected the "ongoing" insulitis stage before severe destruction. Recently, it has been reported that T-helper 1/T-helper 2 (Th1/Th2) imbalance is important in both human (16–18) and murine (19) type 1 diabetes, so the evidence that CD4⁺ cells are present in lesions of insulitis is attractive.

An inverse correlation has been reported between cellular and humoral immunity to certain β-cell antigens, including GAD (20). We have also reported an inverse correlation between serum IFN-γ levels (Th1-type response) and anti-GAD antibody (immunoglobulin G1) levels (Th2-type response) in "typical" type 1 diabetes with HLA-DR4 or -DR9, which are major HLA types in Japanese type 1 diabetic subjects (11). We also showed that a higher

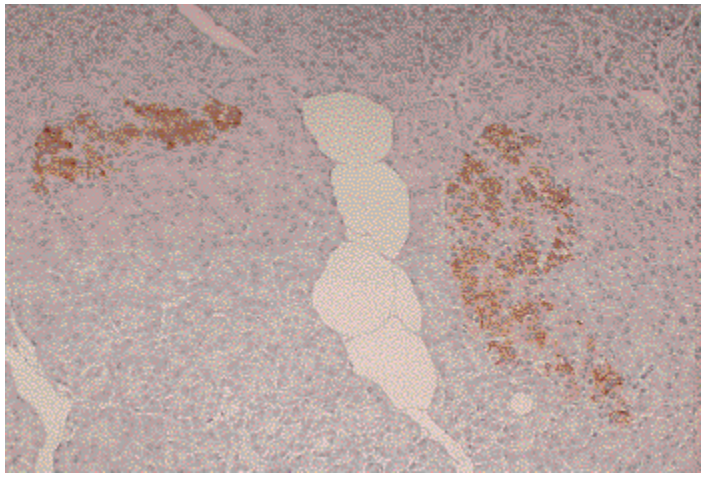

Figure 2—*Staining of insulin in the islet lesion.*

anti-GAD antibody titer positively correlates with age at onset of the disease; higher anti-GAD antibody levels indicate slower disease progression (11). A higher anti-GAD antibody level (low serum IFN-γ level) and slower onset of the disease (65 years of age at onset) were observed in our patient, perhaps reflecting the activation of Th2-type cells against GAD (β-cell antigen).

Some reports have indicated that β-cell mass is reduced in typical type 1 diabetic patients in a ketotic state (21). On the other hand, even though the degree of β-cell destruction seemed to vary in individual islets, we observed that all of the islets examined in the specimen from our patient still contained insulin, and that the proportion of β- versus non–β-cells in patients and age-matched normal control subjects was not significantly different. These findings suggest that the β-cell mass may have been preserved. Thus, to explain the relatively lower urine C-peptide level at the onset and the recovery to the normal range in this case at present, we speculate that the β-cells were not severely destroyed but that β-cell dysfunction may have existed. This view agrees with a report suggesting that β-cell destruction may be a late consequence of the autoimmune process and that β-cell mass seems to be preserved more than previously thought at the onset of the disease in a type 1 diabetes (NOD) model (22).

Finally, this case represents the oldest reported patient with proven insulitis. It is important to recognize the existence of a case like this even in an older patient. We believe that this novel evidence will contribute to the proper classification and treatment of diabetes and to a better understanding of the pathophysiology of type 1 diabetes.

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