L-Asparaginase-Induced Continuous Hyperglycemia With Type 1 Diabetes-Related Antibodies and HLA Genotypes: A Case Study

Yasuhisa Furuta 1, Shigeru Yatoh 1,2,3, Hitoshi Iwasaki 1, Yoko Sugano 1, Motohiro Sekiya 1, Hiroaki Suzuki 1, Hitoshi Shimano 1,4,5,6

1. Department of Endocrinology and Metabolism, Faculty of Medicine, University of Tsukuba, Tsukuba, JPN
2. Community-Based Medicine System, Faculty of Medicine, University of Tsukuba, Tsukuba, JPN
3. Internal Medicine, Toride Community Medical Education Station, University of Tsukuba Hospital / Toride-Kitasoma Medical Association Hospital, Toride, JPN
4. International Institute for Integrative Sleep Medicine, University of Tsukuba, Tsukuba, JPN
5. Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance, University of Tsukuba, Tsukuba, JPN
6. Agency for Medical Research and Development-Strategic Basic Research Program (AMED-CREST), Japan Agency for Medical Research and Development, Chiyoda-ku, JPN

Corresponding author: Shigeru Yatoh, yatou-endo@umin.ac.jp

Abstract

A 19-year-old male presented with fatigue and dyspnea on exertion. He was diagnosed with acute T-cell lymphoblastic leukemia. After following the Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) 2003 protocol that incorporates L-asparaginase (L-Asp) treatment, blood glucose levels became elevated for more than one year and insulin secretion was depleted. Anti-glutamic acid decarboxylase (GAD) and anti-islet antigen 2 (IA-2) antibody levels were both positive, which is rare. The patient’s HLA genotype was sensitive for type 1 diabetes. L-Asp can cause transient hyperglycemia as a side effect. However, cases with the anti-GAD antibody have not been reported in L-Asp-induced diabetes. In summary, L-Asp-induced continuous hyperglycemia might be associated with a type 1 diabetes-related HLA genotype through elevations of anti-GAD and anti-IA-2 antibodies.

Introduction

L-asparaginase (L-Asp) is used as a therapeutic drug for acute T-cell lymphoblastic leukemia [1,2]. Its side effects include hyperglycemia, pancreatitis, and dyslipidemia [3-8]. L-asparaginase-associated hyperglycemia has been reported in 4-20% of pediatric patients receiving Escherichia coli-derived asparaginase for acute lymphoblastic leukemia and in 4-17% of patients receiving Erwinia-derived asparaginase [9]. It can cause diabetic ketoacidosis due to severe insulin deficiency [10,11].

Type 1 diabetes is caused by the progressive autoimmune destruction of pancreatic beta cells producing insulin. The anti-glutamic acid decarboxylase (GAD) antibody is present in 70-80% of patients with new-onset type 1 diabetes [11].

The type 1 diabetes susceptibility HLA-alleles, DRB1*0405, DRB1*0901, DRB1*0802-DQB1*0302, DRB1*0405-DQB1*0401, and DRB1*0901-DQB1*0303, were described in patients with anti-GAD antibody and insulin deficiency in a Japanese population [12,13].

No cases with the anti-GAD antibody have been reported in L-Asp-induced diabetes. Here, we report the first case of L-Asp-induced diabetes, together with the transient appearance of anti-GAD antibody, associated with the type 1 diabetes susceptibility HLA alleles, DRB1*0405/*0802.

Case Presentation

A 19-year-old male experienced fatigue and dyspnea on exertion for two months. His blood count revealed leukocytosis, erythrocytopenia, and thrombocytopenia. As a result of a bone marrow biopsy, he was diagnosed with acute T-cell lymphoblastic leukemia.

The Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) 2003 protocol is outlined in Table 1 [14]. Remission induction therapy, including L-Asp and a large amount of corticosteroid, was initiated. L-asparaginase was only used during the first remission induction therapy. After five weeks, a bone marrow test showed hematological remission.
## Remission induction therapy protocol

| Medication     | Amount               | Day  |
|----------------|----------------------|------|
| Daunorubicin   | 85 mg (50 mg/m²)     | 1–3  |
|                | 51 mg (30 mg/m²)     | 15,16|
| Vincristine    | 2 mg                 | 1,8,15,22 |
| L-asparaginase | 10,000 U (6,000 U/m²)| 8,10,12,20,22,24,26,28 |
| Cyclophosphamide | 1,250 mg (750 mg/m²) | 1,15 |
| Prednisolone   | 100 mg (60 mg/m²)    | 1–14 |

## Consolidation therapy protocol

| Medication     | Amount               | Day  |
|----------------|----------------------|------|
| Cytarabine     | 6,100 mg (4,000 mg/m²)| 1,2  |
| Dexamethasone  | 20 mg                | 1,2  |
| L-asparaginase | 15,000 U (10,000 U/m²)| 3    |

### TABLE 1: GRAALL 2003 Protocol

**GRAALL**: Group for Research on Adult Acute Lymphoblastic Leukemia

Consolidation therapy was then started. The fasting plasma glucose level increased to over 400 mg/dL. Selected biochemical test results are shown in Table 2. Plasma levels of insulin and C-peptide were 2.0 μU/mL and 0.25 ng/mL, respectively. Anti-GAD and anti-islet antigen 2 (IA-2) antibody levels were measured at 13.4 U/mL and 3.1 U/mL, respectively, and samples were therefore considered positive for these two antibodies.
### Lab Parameters

| Lab Parameters | Value | Unit | Lab Parameters | Value | Unit |
|----------------|-------|------|----------------|-------|------|
| WBC            | 900   | /μL  | ALB            | 3.8   | g/dL |
| RBC            | 291 x 10⁴ | /μL  | AST            | 18    | U/L  |
| Hb             | 8.8   | g/dL | ALT            | 64    | U/L  |
| Ht             | 25.9  | %    | LDH            | 106   | U/L  |
| Plt            | 8.5 x 10⁴ | /μL  | γ-GTP          | 28    | U/L  |
| PT             | 15.6  | s (INR 1.33) | Na    | 130   | mEq/L|
| APTT           | 40.9  | s (con 26.9 s) | Cl   | 95    | mEq/L|
| Glucose        | 483   | mg/dL| Ca             | 9     | mg/dL|
| IRI            | 2     | μU/mL| IP             | 4.7   | mg/dL|
| CPR            | 0.25  | ng/mL| BUN            | 18.4  | mg/dL|
| GAD antibody   | 13.4  | U/mL | CRE            | 0.49  | mg/dL|
| IA-2 antibody  | 3.1   | U/mL | CRP            | 0.09  | mg/dL|

### TABLE 2: Laboratory Findings

WBC: white blood cells, RBC: red blood cells, Hb: hemoglobin, Ht: hematocrit, Plt: platelets, PT: prothrombin time, APTT: activated partial thromboplastin time, IRI: immunoreactive insulin, CPR: C-peptide immunoreactivity, GAD antibody: glutamic acid decarboxylase antibody, IA-2 antibody: islet antigen 2 antibody, ALB: albumin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, γ-GTP: gamma-glutamyl transferase, T-Bil: total bilirubin, IP: inorganic phosphorus, BUN: blood urea nitrogen, CRE: creatinine, CRP: C-reactive protein

Intensive insulin therapy was started on a sliding scale. The total amount of insulin given was approximately 60 units per day. An insulin secretory defect was thought to cause hyperglycemia. L-asparaginase induces diabetes mellitus as a side effect [14], and both anti-GAD and anti-IA-2 antibody levels were found to be positive. Since this was not common, we regularly measured C-peptide and anti-GAD and anti-IA-2 antibody levels during the intensive insulin therapy.

The time courses for measured glucose, insulin, and C-peptide levels are shown in Table 3. The fasting C-peptide level was found to gradually recover. The total amount of insulin administered decreased from approximately 60 units to about two units per day.

### TABLE 3: Time courses of glucose, insulin, and C-peptide

L-asp: L-asparaginase, FPG: fasting plasma glucose, CPR: C-peptide immunoreactivity, GXR: insulin glargine

Time courses for GAD and IA-2 antibodies are shown in Figure 1. Both anti-GAD and anti-IA-2 antibody levels decreased over time. The anti-GAD antibody level was considered negative nine months after remission induction therapy was initiated.
In order to evaluate insulin secretion 11 months after remission induction therapy was started, a glucagon test was performed and plasma glucose/insulin/C-peptide levels before and after breakfast, as well as urinary C-peptide, were assessed. These findings are shown in Table 4. Insulin secretion did not become depleted.

| Glucagon test | minutes | 0  | 3  | 6  | 10 | 20 | 30 |
|---------------|---------|----|----|----|----|----|----|
| PG            | mg/dL   | 201| 220| 238| 247| 265| 280|
| IRI           | μU/mL   | 8.4| 22.7| 15.4| 12.2| 7.9| 5.7|
| CPR           | ng/mL   | 1.88| 2.92| 2.76| 2.53| 2.22| 1.84|

| Meal test     | PG (Unit) | IRI (mg/dL) | CPR (μU/mL) | CPI (ng/mL) |
|---------------|------------|-------------|-------------|-------------|
| Before breakfast | 159          | 6.4         | 1.74        | 1.09        |
| After breakfast   | 226          | 12.8        | 2.38        | 1.05        |

The patient was administered because of a relapse of acute T-cell lymphoblastic leukemia. He felt a loss of appetite and fatigue; such stresses subsequently induced hyperglycemia. A computed tomography (CT) scan showed atrophy of the pancreas (Figure 2). Therefore, the pancreatic exocrine function was evaluated. A fecal digestion status test showed fatty stool. A low pancreatic function diagnostic test score of 20.6% (standard: >73.4%) was obtained.
Furthermore, an HLA gene test was performed for a marrow transplant. Incidentally, the patient’s HLA-DRB1, consisting of 04:05 and 08:02, was found to be a susceptible gene for type 1 diabetes [15].

Discussion

L-asparaginase is an enzyme that hydrolyses asparagine to aspartic acid and ammonia, causing a depletion of asparagine in cells. Insulin incorporates three molecules of asparagine; therefore, L-Asp can inhibit its synthesis in pancreatic beta cells to cause transient hyperglycemia and diabetes mellitus [16,17]. The percentage of cases with acute lymphoblastic leukemia in whom hyperglycemia occurs due to L-Asp with steroid therapy is 2.5-23% [18]. Hyperglycemia occurs within 5-10 days after the initiation of L-Asp therapy. Although patients require transient insulin therapy, hyperglycemia improves in almost all cases.

In addition to transient glucose intolerance, some rare cases develop pancreatic diabetes with L-Asp-related pancreatitis. Twenty-one percent of patients with L-Asp-related pancreatitis require insulin therapy in the acute phase and 6% of patients continue insulin therapy for one year [19,20]. However, no L-Asp-related diabetes mellitus cases have been positive for both anti-GAD and anti-IA-2 antibodies.

When we explored a mechanism for how the antibodies had appeared, it became clear the patient had specific HLA genes that made him susceptible to type 1 diabetes mellitus. The relative risk of this genetic combination was estimated to be 15 times [15]. It may be that the patient’s condition made him more susceptible to developing type 1 diabetes mellitus. L-asparaginase subsequently induced pancreatic beta-cell impairment, leading to the leakage of intracellular substances into the blood. As a result of antigen presentation, anti-GAD and anti-IA-2 antibody levels became transiently positive.

Next, we considered how the increase in these antibodies affected the decrease in insulin due to the impairment of pancreatic beta cells.

In Table 3, C-peptide levels seemed to be recovering gradually. The recovery might be mainly due to the resolution of glucotoxicity and functional beta cell impairment induced by L-Asp. However, the results of the glucagon test and assessment of plasma glucose/insulin/C-peptide levels before and after breakfast indicated hyperglycemia and insufficient C-peptide. In spite of the hyperglycemia, urinary C-peptide was only 36 μg/day (Table 4). To assess these results comprehensively, the significantly decreased insulin level after L-Asp administration gradually recovered. However, the plasma glucose level was not normal. We considered that insulin secretion had decreased compared to before L-Asp administration.

We discussed whether the limited decrease of insulin depended on the L-Asp effect or autoimmune
destruction of pancreatic beta cells due to type 1 diabetes mellitus. Compared to a typical course of L-Asp-induced diabetes, it was uncommon that pancreatitis did not occur clinically and insulin therapy was necessary after a year. We, therefore, speculated that the mechanism involved was due to type 1 diabetes mellitus. However, we thought that the speculation was unlikely because type 1 diabetes mellitus usually causes extensive destruction of beta cells and needs the administration of massive insulin permanently. On the other hand, the limited decrease in insulin was explainable if L-Asp had caused pancreatitis. The atrophic pancreas in the CT scan and the decrease in pancreatic exocrine function were consistent with post-pancreatectomy changes. We might have missed an acute phase of pancreatitis.

Conclusions
We describe a unique case of L-asparaginase-induced continuous hyperglycemia with type 1 diabetes-related antibodies. Although diabetes mellitus occurred after the administration of L-Asp, it was found that anti-GAD and anti-IA-2 antibodies, which are indicators of type 1 diabetes mellitus, were transiently high. This could be explained by the presence of a type 1 diabetes mellitus-sensitive HLA gene.

Additional Information
Disclosures
Human subjects: Consent was obtained or waived by all participants in this study. Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Acknowledgements
The first author Y.F. treated the case and wrote this case report with the corresponding author S.Y. The co-authors (H.I., Y.S., M.S., H.S., and H.S.) made suggestions and advised about the mechanism of insulin deficiency through clinical conferences. Especially, H.S. and H.S helped to complete the discussion. All authors read and checked this report.

References
1. Ben Tannous M, Sharif-Akbari B, Ceppi F, et al.: Polymorphisms of asparaginase pathway and asparaginase-related complications in children with acute lymphoblastic leukemia. Clin Cancer Res. 2015, 21:329-34. 10.1158/1078-0432.CCR-14-0508
2. Morimoto T, Hirai K, Fukumura A, Takakura H, Koike T, Shimizu T: Early diagnosis of asparaginase-associated pancreatitis based on elevated serum elastase-1 levels: case reports. Biomed Rep. 2015, 1:651-5. 10.3892/br.2015.108
3. Schmiegelow K, Müller K, Mogensen SS, et al.: Non-infectious chemotherapy-associated acute toxicities during childhood acute lymphoblastic leukemia therapy. F1000Res. 2017, 6:444. 10.12688/f1000research.10768.1
4. Stefanović M, Jazbec J, Lünßgen D, Bulajić M, Löhr M: Acute pancreatitis as a complication of childhood cancer treatment. Cancer Med. 2016, 5:327-36. 10.1002/cam4.649
5. Liu C, Yang W, Devidas M, et al.: Clinical and genetic risk factors for acute pancreatitis in patients with acute lymphoblastic leukemia. J Clin Oncol. 2016, 54:2153-40. 10.1200/JCO.2015.64.5812
6. Kearney SL, Dahlberg SE, Levy DE, Voss SD, Sallan SE, Silverman LB: Clinical course and outcome in children with acute lymphoblastic leukemia and asparaginase-associated pancreatitis. Pediatr Blood Cancer. 2009, 53:162-7. 10.1002/pbc.22076
7. Koprivnikar J, McCloskey J, Faderl S: Safety, efficacy, and clinical utility of asparaginase in the treatment of adult patients with acute lymphoblastic leukemia. Onco Targets Ther. 2017, 10:1413-22. 10.2147/OTT.S106810
8. Raja RA, Schmiegelow K, Frandsen TL: Asparaginase-associated pancreatitis in children. Br J Haematol. 2012, 159:18-27. 10.1111/bjh.12016
9. Hijiya N, van der Sluis IM: Asparaginase-associated toxicity in children with acute lymphoblastic leukemia. Leuk Lymphoma. 2016, 57:748-57. 10.3109/10428194.2015.1101098
10. Gifford G, Milliken S, Greenfield J: Diabetic ketoacidosis secondary to L-asparaginase in acute lymphoblastic leukemia. Intern Med J. 2015, 45:946-8. 10.1111/imj.12216
11. Roberson JR, Raja S, Sheko I, Pui CH, Howard SC: Diabetic ketoacidosis during therapy for pediatric acute lymphoblastic leukemia. Pediatr Blood Cancer. 2008, 50:1207-12. 10.1002/pbc.21505
12. Takeda H, Kawasaki E, Shimizu T, et al.: Clinical, autoimmune, and genetic characteristics of adult-onset diabetic patients with GAD autoantibodies in Japan (Ehime Study). Diabetes Care. 2002, 25:995-1001. 10.2337/diacare.25.6.995
13. Yamashita H, Awata T, Kawasaki E, et al.: Analysis of the HLA and non-HLA susceptibility loci in Japanese type 1 diabetes. Diabetes Metab Res Rev. 2011, 27:844-8. 10.1002/dmrr.1254
14. Huguet F, Leguay T, Raffoux E, et al.: Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. J Clin Oncol. 2009, 27:911-8. 10.1200/JCO.2008.18.6916

15. Kawabata Y, Ikegami H, Kawaguchi Y, et al.: Asian-specific HLA haplotypes reveal heterogeneity of the contribution of HLA-DR and -DQ haplotypes to susceptibility to type 1 diabetes. Diabetes. 2002, 51:545-51. 10.2337/diabetes.51.2.545

16. Whitecar JP, Bodey GP, Hill CS Jr, Samaan NA: Effect of L-asparaginase on carbohydrate metabolism. Metabolism. 1970, 19:581-86. 10.1016/0026-0495(70)90014-4

17. Wolthers BO, Mogensen PR, Frandsen TL, et al.: Insulin-dependent diabetes: a chronic complication to acute pancreatitis in childhood acute lymphoblastic leukemia. Pediatr Blood Cancer. 2019, 66.e27437. 10.1002/pbc.27437

18. Quintanilla-Flores DL, Flores-Caballero MÁ, Rodriguez-Gutiérrez R, Tamez-Pérez HE, González-González IG: Acute pancreatitis and diabetic ketoacidosis following L-asparaginase/prednisone therapy in acute lymphoblastic leukemia. Case Rep Oncol Med. 2014, 2014:139169. 10.1155/2014/139169

19. Wolthers BO, Frandsen TL, Baruchel A, et al.: Asparaginase-associated pancreatitis in childhood acute lymphoblastic leukemia: an observational Ponte di Legno Toxicity Working Group study. Lancet Oncol. 2017, 18:1238-48. 10.1016/S1470-2045(17)30424-2

20. Spraker HL, Spyridis GP, Pui CH, Howard SC: Conservative management of pancreatic pseudocysts in children with acute lymphoblastic leukemia. J Pediatr Hematol Oncol. 2009, 31:957-9. 10.1097/MPH.0b013e5181f9e6a