Celiac Disease Refractory to a Gluten-free Diet?

Leann M. Mikesh,1 Sheila E. Crowe,2 Grant C. Bullock,1 Nancy E. Taylor,1 and David E. Bruns1*

CASE DESCRIPTION

A 75-year-old woman from an outside hospital was referred because of continued signs and symptoms of celiac disease (gluten-sensitive enteropathy) that persisted despite self-reported adherence to a gluten-free diet. The patient reported excessive gas, bowel distension, a 15-pound weight loss over the past few years, insomnia, and a rash over her lower extremities. The patient had required hospitalizations, intravenous fluids, and continuing therapy with corticosteroids for 6 months.

A diagnosis of celiac disease had been made 6 years previously, based on (a) typical gastrointestinal signs and symptoms with negative stool cultures and Clostridium difficile toxin assay, (b) positive serology for celiac disease, (c) unremarkable colonoscopy with normal random biopsy results, and (d) small-bowel biopsy results showing evidence of villous blunting with increased chronic inflammatory cells. At that time, the patient’s laboratory results included antigliadin antibody (AGA) IgG 0.8 AU (<10 AU), anti-AGA IgA 1.1 AU (<5 AU), anti–tissue transglutaminase (tTG) IgA 9.2 AU (<4 AU), and normal total IgA and IgA anti-endomysial antibody (EMA) values. A computed tomographic scan was negative for lymphoma, and an upper gastrointestinal series and small-bowel follow-through barium x-ray were normal. Endoscopic biopsy results obtained during the previous 2 years showed continued villous atrophy with intraepithelial lymphocytes. Shortly before the patient’s referral, repeat biopsies showed villous blunting with increased chronic inflammation, findings confirmed by a gastrointestinal pathologist at our institution.

The patient, a pleasant, frail-looking, elderly woman in no acute distress, was retired and married with 2 adult children. She denied smoking and alcohol use and had no family history of celiac disease, liver disease, or colon cancer. Her medical history was remarkable for placement of a carotid artery stent 5 years earlier. Physical examination was unremarkable except for the presence of a maculopapular rash inconsistent with dermatitis herpetiformis and with dependent distribution over the lower legs.

The patient’s blood pressure was 133/59 mmHg, pulse 51 beats/min, temperature 36.5 °C, and weight 59.4 kg. Laboratory results since her referral included vitamin B12 245 ng/L [reference interval (RI), 251–911 ng/L], iron 370 μg/L (RI, 400–1450 μg/L), anti-tTG IgA 13 AU (RI, 0–20 AU), and 5’nucleotidase 22.1 U/L (RI, 4.0–11.5 U/L).

The patient met with a nutritionist and implemented recommended dietary changes to eliminate gluten. Her symptoms temporarily improved, with a return to normal bowel function, but after a short time her symptoms recurred. Results of further tests excluded conditions known to complicate or coexist with celiac disease, including bacterial overgrowth, microscopic colitis, and lactose intolerance. Because the patient’s symptoms were refractory to treatment and required prolonged, continued use of corticosteroid therapy, esophagogastroduodenoscopy with duodenal biopsies was performed, and formalin-fixed small-bowel biopsy tissue samples were sent to the molecular diagnostic laboratory for additional testing.

DISCUSSION

CELIAC DISEASE

Celiac disease is a T-cell driven, multifactorial chronic inflammatory disorder of the small intestine characterized by mucosal inflammation, villous atrophy, and crypt hyperplasia; it has a prevalence of approximately 1% in the population. Among autoimmune diseases, celiac disease is unique in that an environmental trigger (gluten) and an autoantigen (tissue transglutaminase) have been identified (1).

The main dietary sources of gluten are wheat, rye, barley, and oats, but the gluten in oats has not been found to contribute to celiac disease. Gluten is broken down into smaller peptides by gastric acid and digestive enzymes. In the intestine, tTG converts glutamine to glutamic acid, thereby increasing the affinity of the binding of gluten peptides in the cleft of HLA class II molecules. The modified peptides are inappropriately
recognized by helper T cells, perhaps because of molecular mimicry of microbial peptides The identity of these immunogenic peptides has been determined (2–4). Most individuals with celiac disease express HLA-DQ2 (95% of patients), and the others typically express HLA-DQ8. The presence of HLA-DQ2 and/or DQ8 alone, however, is not sufficient for disease, which is thought to involve other contributing factors such as additional genetic loci, stress, inflammation, and infection. The key treatment for celiac disease is lifelong adherence to a strict gluten-free diet.

**DIAGNOSIS OF CELIAC DISEASE**

The diagnosis of celiac disease is based on concordance of serological tests, small-bowel biopsy, and resolution of symptoms on withdrawal of gluten from the diet (5, 6). Serological testing for celiac disease includes anti-EMA IgA and anti-tTG IgA and IgG. Anti-AGA tests are no longer recommended because of their lower sensitivity and specificity (anti-AGA IgA sensitivity 75%–95%, specificity 80%–95%) (7); anti-AGA IgG sensitivity 57%–100%, specificity 47%–94%) (8).

In the anti-EMA test, antibodies from the patient’s serum bind to connective tissue surrounding smooth muscle cells of either monkey esophagus or human umbilical cord and are detected by immunofluorescence. Identification of EMA as tTG led to the development of anti-tTG immunoassays. The first assays used guinea pig tTG; current assays use human tTG, either native (from erythrocytes) or recombinant. The anti-tTG test is easier to perform and more cost-effective than the anti-EMA test, and results are objective and quantitative. The test approaches 100% specificity and >90% sensitivity for celiac disease in a variety of clinical settings and populations (see Table 1) (7). A test for antibodies against a 9 amino-acid deamidated gliadin peptide has recently become commercially available, but few studies of its diagnostic accuracy have been published.

The gold standard for diagnosis is histopathologic assessment of 4–8 mucosal biopsy specimens of the small bowel obtained while the patient is on a diet containing gluten.

**CAUSES OF FAILURE TO RESPOND TO TREATMENT**

The case patient is among a small proportion of individuals with celiac disease whose illness does not respond to a gluten-free diet. The 3 main causes of treatment failure are (i) inadvertent or intentional failure to adhere to a strict gluten-free diet; (ii) other complicating or coexisting conditions such as small-bowel bacterial overgrowth, lactose intolerance, or microscopic colitis; and (iii) disease refractoriness to a gluten-free diet. The patient described here was compliant with the diet, and diagnostic testing revealed no evidence of lactose intolerance, microscopic colitis, small bowel bacterial overgrowth, ulcerative jejunitis, or lymphoma. These findings suggest a diagnosis of refractory celiac disease, which is characterized by persistent villous atrophy with an increase of intraepithelial lymphocytes in the small bowel while the patient is on a long-term gluten-free diet. In both responsive and refractory celiac disease, celiac antibodies usually decrease with dietary therapy (as observed in this case) and remain within reference intervals unless individuals are reexposed to gluten.

Two types of refractory celiac disease occur and are differentiated by the type of T-cell populations in the intestinal mucosa, which are polyclonal in type I disease and clonal in type II disease (9). Although the presence of this clonal T-cell population is termed “cryptic intraepithelial lymphoma,” this finding does not imply a diagnosis of a malignant process, although enteropathy-associated T-cell lymphoma develops in a subset of these patients.

**TCRγ GENE REARRANGEMENT**

In the case patient, a T-cell receptor γ locus (TCRγ) gene rearrangement assay was the molecular test performed on the intestinal biopsy specimens to test for the presence of a clonal population of T cells.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive likelihood ratio</th>
<th>Negative likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA-EMA-ME</td>
<td>80–90%</td>
<td>99.5%</td>
<td>160–180</td>
<td>0.1–0.2</td>
</tr>
<tr>
<td>IgA-EMA-HU</td>
<td>92.5%</td>
<td>99.6%</td>
<td>231</td>
<td>0.1</td>
</tr>
<tr>
<td>IgA-tTG-GP</td>
<td>85–95%</td>
<td>95.4%</td>
<td>18–21</td>
<td>0.05–0.16</td>
</tr>
<tr>
<td>IgA-tTG-HR</td>
<td>90.2%</td>
<td>95.4%</td>
<td>20</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* Adapted from (7). Values shown are from a metaanalysis of mixed populations of adults and children in a variety of clinical settings. EMA, endomysial antibody; ME, monkey esophagus; HU, human umbilical cord; tTG, tissue transglutaminase; GP, guinea pig; HR, human recombinant.
Functional human TCRγ is encoded by the random rearrangement of 1 of 10 variable (V) segments and 1 of 5 joining (J) segments of the TCRγ gene. During T-cell maturation in the bone marrow, the (V) and (J) gene segments are randomly recombined to form a functional TCRγ chain.

The TCRγ chain gene segments are located on chromosome 7p14, and every T cell carries 2 alleles (paternal and maternal) of this locus. During T-cell development one or both alleles undergo rearrangement, so a clonal T-cell population carries 1 (monallelic) or 2 (biallelic) rearranged TCRγ chain genes.

In the test for clonal T cells, pairs of specific PCR primers target the conserved regions flanking the (V) and (J) gene segments. Nonrearranged (V) and (J) segments in the germ line configuration are far apart, and therefore do not give rise to PCR products. A PCR product will arise only from rearranged (V) and (J) segments. Each specific (V) and (J) rearrangement produces a PCR product of a characteristic size. Individuals without celiac disease will have many different T cells, each with a specific TCRγ gene distribution of peak sizes and heights without any dominant fluorescent signals. (B), The patient’s intestinal biopsy tissue reveals a predominant TCRγ gene rearrangement amplified by primers targeting Vγ10 to Jγ1.1/2.1 (175–195 nt, blue). The predominant amplicon (192 nt) produced a fluorescent peak height intensity more than 3 times greater than the mean polyclonal background peak height intensity.

Clinical Case Study

Fig. 1. TCRγ Gene Rearrangement Study.
The PCR-based TCRγ gene clonality assay shows the products of 4 amplification reactions ranging in size from 145–255 nucleotides (nt) targeting the TCR Vγ 1–8 + 10 and 4 of the 5 Jγ gene segments. The 4 regions are Vγ 1–8 to Jγ1.1/2.1 (230–255 nt, blue); Vγ1–8 to Jγ1.3/2.3 (195–230 nt, green); Vγ10 to Jγ1.1/2.1 (175–195 nt, blue); and Vγ10 to Jγ1.3/2.3 (145–175 nt, green). Every cell in a clonal population of T cells carries the same (V) and (J) rearrangements, producing a predominant peak of a specific size with greater fluorescent intensity than the background polyclonal population of T cells. This finding indicates that one population of T cells has proliferated and outnumbered all other T cells. The size distribution of the PCR products is limited by the specific primer pair used and shows a gaussian distribution across this primer-defined range of sizes. One of the primers is fluorescently tagged, allowing detection after capillary gel electrophoresis. The intensity of fluorescence indicates the relative amount of any given PCR product compared to the rest of the population of PCR products. Size markers are shown in red. (A), The polyclonal control produces the pattern expected for a polyclonal TCR distribution. The polyclonal population of fluorescent peaks shows a random distribution of peak sizes and heights without any dominant fluorescent signals. (B), The patient’s intestinal biopsy tissue reveals a predominant TCRγ gene rearrangement amplified by primers targeting Vγ10 to Jγ1.1/2.1 (175–195 nt, blue). The predominant amplicon (192 nt) produced a fluorescent peak height intensity more than 3 times greater than the mean polyclonal background peak height intensity.
Clinical Case Study

POUNTS TO REMEMBER

- Celiac disease has a prevalence of 1%, and diagnosis is based on concordance of serologic tests, small bowel biopsy, and resolution of symptoms upon withdrawal of gluten from the diet.
- Wheat, rye, and barley are the main dietary sources of gluten that contribute to celiac disease. The gluten in oats does not seem to contribute to celiac disease.
- Testing for anti-human tTG antibodies provides a sensitivity approaching 100%. Antigliadin antibody testing is no longer recommended because of low sensitivity and specificity.
- Several possible explanations account for patient failure to respond to a gluten-free diet, including nonadherence, other coexisting conditions (such as small bowel bacterial overgrowth, lactose intolerance, and microscopic colitis), and the presence of type I or II refractory celiac disease.
- An appropriate test for refractory celiac disease for which adherence to a gluten-free diet has been verified is a TCR gene-rearrangement study to determine if a prominent T-cell clone is present in the intestinal mucosa.

PROGNOSIS AND TREATMENT OF TYPE II REFRACTORY CELIAC DISEASE

The 5-year survival for type II refractory celiac disease is <50%, with the most common causes of death being T-cell lymphoma and infection. Treatment options include corticosteroids and immunosuppressive agents such as thiopurines and infliximab. There is concern that immunosuppressive therapy promotes progression to lymphoma, but no data confirm this risk. Therapies under investigation include antibody to IL-15, a cytokine that leads to enhanced enterocyte killing in celiac disease (10), and stem cell transplantation.

Given the results of the TCR studies, repeated confirmation of the patient’s strict adherence to a gluten-free diet, and the patient’s continued dependence on steroids for a period of several months, immunosuppressive therapy was initiated with 6-mercaptopurine with the goal of decreasing or eliminating the need for corticosteroid therapy. The patient was able to discontinue corticosteroids and at the time of this report was doing well on 6-mercaptopurine alone.

Grant/funding Support: L.M.M.’s postdoctoral training in clinical chemistry and laboratory medicine is supported by a Past Presidents’ Scholarship from the Van Slyke Foundation of the American Association for Clinical Chemistry. G.C.B. is supported by a Ruth L. Kirschstein National Research Service Award 1F32HL086046-01. We thank the Department of Pathology for additional support of L.M.M.

Financial Interests: None declared.

References


Commentary

Robin G. Lorenz

The gold standard for diagnosis of celiac disease (CD) requires both a duodenal biopsy showing villus blunting, crypt hyperplasia, and increased numbers of intraepithelial lymphocytes (IELs) and a subsequent small intestinal biopsy that shows resolution of these histological findings after the patient is put on a gluten-free diet (1 ). The development of new serological tests, however, have resulted in the current diagnostic standard of IgA anti-tissue transglutaminase (tTG), which is the autoantigen responsible for the development of endomysial antibodies.

In this case, the patient’s original diagnosis was by a biopsy and IgA anti-tTG. She was refractory, however, to her gluten-free diet (a situation that could be
attributable to nonadherence to the diet). When this possibility is ruled out, a more concerning reason for a poor clinical response is that the patient may have a serious complication of celiac disease, enteropathy-associated T-cell lymphoma (EATL) or refractory CD. EATL is a clonal proliferation of IELs that historically has been diagnosed on the basis of biopsy and immunohistochemical analysis to determine the presence of abnormal T cells in the intestinal epithelium. This case study is one of the first to investigate the use of polymerase chain reaction (PCR) for T-cell receptor (TCR) gene rearrangements in EATL/refractory CD and demonstrates that this approach will detect a clonal rearrangement in intestinal biopsies. It is critical to note, however, that oligo- or monoclonal IEL populations can also be detected in the large majority of refractory CD (both type I and type II), and therefore differentiation between EATL and refractory disease is of limited use (2). Immunohistochemical analysis for T-cell surface receptors (CD3 and CD8) and histological appearance are more useful in the diagnosis of EATL or refractory CD. Serology is useful only in the initial diagnosis.

Grant/funding Support: None declared.

Financial Disclosures: None declared.

References


Commentary

Susan H. Barton and Joseph A. Murray*

Although refractory celiac disease (RCD) is frequently suspected, alternative or additional diagnoses can often explain the patient’s symptoms. First, it is important to carefully review the original diagnosis, especially the biopsy slides and serology. The absence of the specific gene pairs associated with CD risk, DQA1*05:DQB1*02 (DQ2) or DQA1*03:DQB1*0302(DQ8), makes CD unlikely. RCD is categorized into type I (polyclonal phenotype) and type II (clonal expansion of an aberrant intraepithelial T-cell population). The monoclonal phenotype can be detected by immunohistochemical analysis of intraepithelial lymphocytes, which will have cytoplasmic CD3 but lack the typical surface markers of T cells, including CD8, CD4, and T-cell receptor–βF1. T-cell receptor gene rearrangement analysis by PCR on extracted DNA from intestinal biopsies is an alternative method of identifying a T-cell clone, as in this case report. Although sufficient DNA for PCR can usually be obtained from fixed biopsies, we find that fresh frozen samples have a better yield of DNA, making possible Southern blotting in addition to the sensitive but less specific PCR technique. It also should be noted that DNA extraction destroys the tissue blocks, so immunohistochemical analysis should be performed first. Identification of clonal transformation of intraepithelial lymphocytes by flow cytometry has recently been used as an alternative method for diagnosis.

The diagnosis of type II RCD has significant clinical implications. T-cell clonal transformation is typically viewed as an initial step along a continuum leading to overt enteropathy-associated T-cell lymphoma (EATL). Development of EATL is common among RCD II patients and is associated with a high mortality rate. The use of immunosuppressive drugs in RCD II patients is controversial because of the theoretical risk of accelerating the transformation to lymphoma. A recent aggressive approach using myeloablative chemotherapy followed by autologous stem cell support has been used in patients with RCD II with early results that appear encouraging. More recently, budesonide has been shown to improve overall clinical symptoms among both RCD I and II groups and to minimize the adverse side effects associated with chronic immunosuppression. Despite treatment, patients with RCD II frequently clinically deteriorate from complications of severe malnutrition. As aptly illustrated by Mikesh et al., nonresponsive celiac disease is a challenging condition.

Grant/funding Support: S.H.B. is supported by the NIH training grant T32 DK07198. J.A.M. is supported by NIH grants DK57892 and 071003. Financial Disclosures: J.A.M. has been a consultant to Astra Zeneca, Alvine Inc., and Novartis and an investigator for Alba Therapeutics and Dynagen Inc.

References