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CASE REPORT

Erythrocytic transglutaminase inhibition hemolysis at presentation of celiac disease

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Abstract

Celiac disease (CD) is a common autoimmune condition. Previously it was considered to be a rare childhood disorder, but is actually considered a relatively common condition, present at any age, which may have multiple complications and manifestations. Hematological disorders of the disease are not uncommon. Among these disorders, the most frequently reported are anemias as a result of iron deficiency, often associated with folate and/or B12 deficiency. Anemias caused by hemolysis are very rarely reported in celiac patients. An 11-year-old girl with a previous uneventful medical history presented with severe hemolytic anemia. Hemolysis was Coombs negative, accompanied by inappropriate low reticulocyte count, despite exaggerated bone marrow hyperplasia of the erythroid precursors which showed normal maturation. Serology for recent infections, including Epstein-Barr virus, parvovirus B19, cytomegalovirus and mycoplasma, were all negative. Levels of serum IgA, IgG and IgM, were all within normal ranges for age. Screening for anti-DNA, antinuclear, antineutrophil cytoplasmic, antimicrosomal, antithyroglobulin, and antimitochondrial antibodies and lupus anticoagulants, was negative. She was also negative for human immunodeficiency virus. Conventional therapy with corticosteroids and intravenous immunoglobulin failed. CD was serendipitously discovered upon screening for anti-tissue transglutaminase autoantibodies. The disease was confirmed by biopsy of the small intestine mucosa. The patient recovered with gluten-free diet. A unique case of CD is presented. CD should be serologically screened in each patient with Coombs negative "immune" hemolytic anemia, particularly if accompanied by "reticulocytopenia". A new hemolytic mechanism and very speculative explanation for "reticulocytopenia" are discussed.

Key words: Celiac disease; Tissue transglutaminase; Antibodies; Hemolytic anemia; Gluten free diet

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INTRODUCTION

Celiac disease (CD) is a common autoimmune condition, induced by the intake of prolamines, alcohol soluble proteins, rich in glutamine and proline present in wheat, barley and rye, in genetically susceptible persons. Histologically, the disease produces a spectrum of upper small intestinal mucosa changes, ranging from an increase in

the number of intraepithelial lymphocytes to mucosal remodeling with crypt hyperplasia and flattening of the villi. Contrary to common belief this disorder is a systemic protean disease, rather than merely a pure digestive dysfunction. Previously, it was considered to be a rare childhood disorder, but is actually considered a relatively common condition, present at any age, and may have multiple complications and manifestations[1]. Among these, hematological disorders at presentation of the disease, and/or as manifestations during the course of the disease are not uncommon. The most frequent are anemias, including iron-deficiency anemia often associated with folate and/or B_{12} deficiency[2]. Other, less common hematological disorders seen in CD are leucopenia/neutropenia, thrombocytopenia, thrombocytosis, and vitamin K deficiency, manifested as coagulopathy and/or thromboembolism[2]. Hemolytic anemia, as an integral part of the clinical picture of CD, is extremely rare. We describe a child whose CD presented with severe hemolytic anemia with inappropriate reticulocytosis. A new mechanism of hemolysis, specific for CD and a very speculative explanation for "reticulocytopenia" are discussed.

CASE REPORT

An 11-year-old girl, with no previous gastrointestinal or other complaints, presented with acute, severe hemolytic anemia. This was preceded by slight flu-like illness of 2 d duration. On admission she was found to be pale, with mild icteric discoloration of the sclera. She was above the 25th percentile for weight and height. The spleen was slightly enlarged. Initial laboratory testing disclosed an elevated sedimentation rate of 100/146, hemoglobin 4.3 g/L, red blood cell count 1.5×10^{12}/L, and white blood cell count 9.8×10^{6}/L. A peripheral blood smear showed normal white cells and differential, normochromic and normocytic red cells. Neither abnormal red cells, nor normoblasts were seen. The platelet count was 355×10^{9}/L, mean corpuscular volume 83 fL, reticulocyte percentage 3.0%, total bilirubin level 46 μmol/L (normal range 1.7-20.0 μmol/L), direct bilirubin level 13 μmol/L (normal range 1.7-8.6 μmol/L), lactate dehydrogenase 980 U/L, slightly elevated serum transaminases activities (alanine transaminase 67 U/L, aspartate transaminase 72 U/L), and almost undetectable haptoglobin level (< 0.07 g/L). Bone marrow examination revealed hyperplasia of the erythroid precursors with normal maturation. Serology for recent infections, including Epstein-Barr virus, parvovirus B19, cytomegalovirus and mycoplasma, were all negative. A direct antiglobulin test (DAT) with anti-IgG, anti-IgM and anti-C3b was negative. An indirect antiglobulin test was also negative. The ceruloplasmin level was 46.1 g/L. Levels of serum IgA, IgG and IgM, were all within the normal ranges for age. Screening for anti-DNA, antinuclear, antineutrophil cytoplasmic, antimicrosomal, antithyroglobulin, and antimitochondrial antibodies and lupus anticoagulants, was negative. She was also negative for human immunodeficiency virus.

A diagnosis of idiopathic DAT-negative autoimmune hemolytic anemia (AIHA) was established. For the first 7 d, in expectation of spontaneous recovery, she was treated with transfusions of red packed cells only. Hemolysis was so severe that she had to receive at least 500 mL of red packed cells daily to maintain the hemoglobin level just above 50 g/L. On the 7th day, 3 mg/kg per day of prednisone was started with 1 g/kg of intravenous immunoglobulin (IVIG) on 2 consecutive days. She did not improve during the following 14 d. Then we started to taper prednisone, and serendipitously[3] the patient's blood was sampled for anti-tissue transglutaminase antibodies (anti-tTGAbs) and a gluten-free diet (GFD) was introduced. A gradual improvement ensued. The need for blood transfusion started to decrease 7 d after introduction of the GFD and a steady increase in hemoglobin level followed, accompanied by exaggerated reticulocytosis (> 20%). Ten days after blood sampling for anti-tTGAbs testing, we were informed that the anti-tTGAb titer was elevated (45.8 U/mL, normal < 8 U/mL). Thereafter a peroral intestinal enterobiopsy was performed. CD grade Ⅲ was confirmed. Now, 5 years later, on a GFD, she has normal development, normal blood counts, and normal anti-tTGAbs titer.

DISCUSSION

There is no doubt that our patient has CD, which was previously unrecognized. The patient had an uneventful medical history, until a severe, life-threatening hematological disorder appeared. There is also no doubt that the nature of the hematological disorder was anemia caused by a hemolytic mechanism. Until recently we thought that the patient had classical AIHA with negative DAT. AIHA is very rare event in CD and in the general population as well[4]. AIHA is not even included in the hematological spectrum of CD[2], despite the existence of 4 well documented reports of patients having CD and AIHA, (2 had Evans syndrome)[5-7]. These patients, contrary to the presented case were: (1) symptomatic before CD was diagnosed; (2) their hemolysis was DAT positive suggesting that the hemolysis was IgG and/or C3b mediated; (3) hemolysis in these patients was accompanied by exaggerated reticulocytosis; and finally (4) each of these patients responded to corticosteroid treatment. Our patient was (1) asymptomatic before hemolysis appeared; (2) hemolysis was resistant to corticosteroids and IVIG; (3) DAT was negative, meaning that the hemolysis was not mediated by IgG, IgM or C3b; and finally (4) despite the large number of erythroid precursors in the patient's bone marrow, reticulocytes were only marginally elevated (3%).

In an attempt to explain these unusual phenomena in our patient, like The Three Princes[3], we set out on a trip, and kept making unexpected discoveries along our way through the medical literature. Thus, we reached the wonderful world of transglutaminases (TGs)[8], especially the world of tissue transglutaminase (tTG), and their "enemies", the anti-tTGAbs[9]. Anti-tTGAbs found in CD[9]

have special properties which may play a role in the disease, at both local and systemic levels, reflecting the role of tTG in many crucial biological processes. It was shown that anti-tTG-Abs from celiac patients inhibit the enzymatic activity of human tTG both *in vitro*, and *in situ*[10]. Very recent studies showed that *in vivo* targeting of tTG in CD also occurs in the form of *in situ* endomysial, reticulin, and jejunal subepithelial anti-tTGAbs binding[11]. Furthermore, the same study[11] showed IgA deposition on extracellular tTG in the liver, lymph nodes, and muscles indicating that the CD autoantigen is widely accessible to the intestinally-produced circulating autoantibodies throughout the body.

Finally, we were able to elucidate the mystery of our patient. Anti-tTGAbs were produced in her small intestine upon continuous exposure to gluten and distributed *via* the circulation throughout the body. They targeted the erythrocytic TG, so called band 4.2[12] and disabled its anchor function. As a consequence, the patient's erythrocytes became fragile and hemolysis ensued in the very hostile spleen environment (acidosis, hypoglycemia, slowed circulation).

This case should not be classified as classical IgG or C3b mediated AIHA. It should be rather considered as a "celiac type of immune hemolysis", because we believe it was mediated by autoantibodies, which were generated in the intestinal mucosa in gluten intolerance and exerted an anti-tTG activity. This kind of hemolysis could be designated as an enzymatic inhibition induced hemolysis. One could argue that the hemolysis in our patient could be IgA mediated. Unfortunately, at the time in Serbia kits for anti-IgA DAT were not available. Because our patient was not IgA deficient, it could be presumed that the patient's circulating anti-tTGAbs which belong to IgA, targeted the erythrocytic TG (band 4.2). One can also argue that our patient could have AIHA with a low titer of anti-erythrocytic antibodies to explain the negative Coombs test. Having in mind the severity of the hemolysis, we can argue with the question: can low anti-erythrocyte autoantibodies cause such severe hemolysis?

The failure of our patient to respond to corticosteroids and IVIG could be explained by the fact that the patient was fed, i.e. continuously challenged, with gluten and the production of anti-tTGAbs was unrestrained, until the introduction of a GFD.

Finally, we will try to explain the most mysterious phenomenon of our patient and that is the inappropriate low reticulocytes (3%), despite the exaggerated erythroid bone marrow hyperplasia which ensued as a physiologic answer to severe hemolysis.

Fibronectin is a glycoprotein associated with the extracellular matrix of many tissues[13] and is a major component of the interstitial matrix in the bone marrow[14]. Fibronectin is tightly bound to the surface of many cells, including bone marrow precursors. Migration of mature reticulocytes from the bone marrow into the circulation is associated with the loss of fibronectin adhesion from the marrow precursors (mature reticulocytes). This process is probably physiologically mediated by at least 2 bone marrow tTGs. The first one is cell surface tTG, a ubiquitously expressed, potent integrin-binding adhesion coreceptor involved in the binding of cells to fibronectin[15]. Its role would be retention of early erythroid marrow precursors in the bone marrow hematopoietic nests, until their full maturation. After that another tTG, an extracellular tTG, would modulate fibronectin adhesion affinity. This tTG switches fibronectin adhesion from the mature reticulocyte membrane toward the extracellular matrix, *via* tight cross-linking of fibronectin with extracellular matrix proteins[16]. Thus, reticulocytes are liberated and migrate into the circulation.

We suggest that, in our patient, anti-tTGAbs targeted this extracellular bone marrow tTG, and fibronectin adhesion affinity could not be switched, which resulted in trapping of reticulocytes in the bone marrow. This is a very speculative explanation. We cannot offer any other explanation for the low reticulocyte count in our patient, despite the bone marrow erythroid hyperplasia.

This case represents one of the many atypical presentations of CD, and the first one whose CD started with hemolytic anemia. CD should be serologically screened in every patient with DAT-negative immune hemolytic anemia, particularly if accompanied by "reticulocytopenia". This case also confirms the value of antibody screening in early diagnosis of CD. Finally, we believe that we have probably described a new mechanism of hemolysis, which we would designate as an "enzymatic inhibition hemolysis".

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