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CASE REPORT

A Patient with CTLA-4 Haploinsufficiency with Multiple Autoimmune Presentations: A Case Report

Fatemeh Zaremehrjardi[1#], Leila Baniadam[1#], Farhad Seif[2,3], Saba Arshi[1], Mohammad Hassan Bemanian[1], Sima Shokri[1], Afshin Rezaeifar[1], Morteza Fallahpour[1*], Mohammad Nabavi[1*]

[1]Department of Allergy and Clinical Immunology, Rasoul Akram Hospital, Iran University of Medical Sciences, [2]Department of Immunology and Allergy, Academic Center for Education, Culture, and Research, [3]Neuroscience Research Center, Iran University of Medical Sciences, Tehran, Iran

ABSTRACT

Increased susceptibility to autoimmunity, malignancy, and allergy in addition to recurrent infections are the main characteristics suggesting primary immunodeficiency diseases (PID). CTLA-4 is predominantly expressed on activated T-cells and regulatory T-cells, which can bind to CD80/CD86 molecules on antigen presenting cells as a negative regulator. In this article, we describe a patient with heterozygous CTLA-4 mutation who presented with multiple autoimmunities. A 24-year-old male patient from consanguineous parents referred to our clinic with multiple autoimmunities. His past clinical history revealed alopecia areata at four years old and subsequently, he developed Evans syndrome, type 1 diabetes mellitus, hypothyroidism, and chronic diarrhea while chronic rhinosinusitis and cytomegalovirus (CMV) colitis were the only infectious manifestations. Immunologic investigations revealed: low B cell count, abnormal Lymphocyte transformation test (LTT) to phytohemagglutinin (PHA) and hypogammaglobulinemia. Although all available treatments such as Intravenous Immunoglobulin (IVIG) therapy, immunosuppressive drugs, and antibiotic therapy were applied, diarrhea was not controlled due to colitis, which remained challenging and made us seek for genetic study. Whole exome sequencing showed heterozygous variant CHR2.204,735,635 G>A in the CTLA-4 gene, which was confirmed by the Sanger method. CTLA4 haploinsufficiency leads to autoimmune disorders, recurrent respiratory infections, hypo-gammaglobulinemia, lymphoproliferation with organ infiltration and lymphocytic interstitial lung disease.

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Keywords: Abatacept, CTLA-4 Deficiency, Haploinsufficiency, Immunodeficiency, Multiple Autoimmunities

*Corresponding authors: Dr. Morteza Fallahpour, Department of Allergy and Clinical Immunology, Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran, e-Mail: fallahpour.morteza@yahoo.com and Dr. Mohammad Nabavi, Department of Allergy and Clinical Immunology, Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran, e-Mail: mnabavi@yahoo.com
# These authors contributed equally as the first author.

INTRODUCTION

Primary immunodeficiency disorders (PID) are routinely characterized with susceptibility to infections; however, impaired activity of the immune system and immune dysregulation, which lead to autoimmunity, are among other features of PID (1-3). T cells actively contribute to immune responses (4). Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is predominantly expressed on activated T-cells and regulatory T-cells (5,6). CTLA-4 is a negative regulator while CD28 is a positive regulator that can competitively bind to two costimulatory molecules, namely CD80 and CD86, which are exhibited on antigen presenting cells (APC) either to inhibit or activate immune responses, respectively. Moreover, CTLA-4 plays pivotal roles regarding peripheral tolerance against self- and non-self-antigens, participating in self-tolerance and autoimmune diseases, respectively. Therefore, in comparison to CD28, CTLA-4 has a higher affinity for CD80/CD86 and can effectively prevent T-cell co-stimulation (6,7). Investigations have shown that CTLA-4 Haploinsufficiency in human (8) or CTLA-4 knockout in mice (9) can cause impaired immune responses. CTLA4 deficiency is known as an immune dysregulation syndrome (10). In a patient with primary immunodeficiency with multiple autoimmune manifestations, it is important to identify CTLA4 deficiency when it comes to applying a new treatment with Abatacept (11). Abatacept (Orencia) is a fusion protein constructed by an IgG1 Fc region linked with the extracellular domain of CTLA-4 (CTLA-4-Ig) (12). Here, we describe a patient with heterozygous CTLA-4 mutation who presented with multiple autoimmunities.

CASE REPORT

In 2014, a 24-year-old male patient referred to the Allergy and Clinical Immunology Clinic, Rasoul Akram Hospital for further evaluation of multiple autoimmunities. The patient was born from consanguineous parents. One of his step uncles was diagnosed with leukemia and CNS infection and another one had autoimmunities such as alopecia, vitiligo, and chronic idiopathic thrombocytopenic purpura (ITP). One of his step aunts had pancytopenia and myelodysplastic syndrome whereas another one was under treatment with intravenous immunoglobulin (IVIG) due to common variable immune deficiency. Apart from his low weight and the foregoing complications, clinical examinations were insignificant. His complaints started with alopecia areata when he was four years old. At 14 of years' age, he had an episode of chronic ITP, which was treated with corticosteroids and IVIG. After the age of 17, he suffered from chronic diarrhea. Severe weight loss and electrolyte imbalance led to multiple admissions, and after several paraclinical and laboratory assessments, he was diagnosed with enteropathy. Upper gastrointestinal endoscopy and intestinal biopsies were performed to evaluate celiac disease, which did not establish this diagnosis. At the age of 18, he was hospitalized following a diagnosis of Evans syndrome and received IVIG and two courses of Rituximab. At 21, he had a cholecystectomy probably due to autoimmune hemolytic anemia episodes. A year later, when he was 22 years old, he was diagnosed with hypothyroidism for which he received treatment. At 23 years of age, he had an episode of severe diarrhea. Colon biopsies revealed cytomegalovirus (CMV) infection, treated with ganciclovir for three months. At the age of 25, he developed diabetes mellitus type 1 for which medical treatment was considered. Chronic sinusitis was a

significant infection that he mentioned. We performed immunological tests on this subject in our clinic (Table 1).

Table 1. Lymphocyte subset percentage (count).

Lymphocyte subset	Percentage (count)	Normal range
CD3	84 (1431)	73 (1000-2200)
CD4	38 (655)	41 (530-1300)
CD8	42 (172)	26 (330-920)
CD19	3.9 (67)	14 (110-570)
CD16/56	10 (172)	9 (70-480)

The flow cytometry results revealed a significant reduction in $CD19^+$ B cells, low levels of total IgG and IgA, and a normal IgE level (table 2). His antibody response to diphtheria and pneumococcal vaccines was impaired. Peripheral blood mononuclear cell (PBMC) proliferation against PHA and BCG was also reduced.

Table 2. Serum immunoglobulin levels.

Immunoglobulin	Serum level	Normal range
IgG	300 mg/dl	767-1590
IgM	68 mg/dl	37-286
IgA	6 mg/dl	61-356
IgE	1 IU/ml	<257

He was diagnosed with combined immunodeficiency. Hence, IVIG therapy was initiated and chronic sinusitis was treated with appropriate antibiotics. Whole exome sequencing followed by Sanger confirmation identified heterozygote variant (Chr. 2q33.2) in the CTLA-4 gene with single nucleotide change c.436G>A in exon 2, leading to missense variant p.G146R and not shared with his mother. His father refused a genetic study. For the next four years, the patient received IVIG therapy every four weeks; however, chronic diarrhea persisted, and he had two episodes of severe diarrhea; this led to electrolyte imbalance and a significant weight loss, both treated with conservative therapy.

DISCUSSION

The over activity of the immune system results in autoimmunity and inflammatory complications different from infections, which are indicators of immune deficiency (13). To date, a few underlying causes of immune dysregulation have been elucidated. CTLA-4 deficiency was detected in the cohorts of patients diagnosed with antibody deficiency and severe immune dysregulation (8,14). CLTA-4 is a transmembrane protein expressed mostly by T lymphocytes. It is similar to the stimulatory receptor CD28 observed on T cell membrane. CD28 engagement leads to T-cell activation, cytokine production, and memory T-cell differentiation. Despite sharing the same

ligands, CTLA-4 and CD28 function in opposite ways; furthermore, their interaction balances both the effective immune response to pathogens and the regulatory autoimmune response against self-tissues. Therefore, CTLA-4 mutation leads to lower expression levels of its product over T regulatory ($CD4^+Foxp3^+$ T cells), leading to autoimmune dysregulation and autoimmunities. Many CTLA-4 mutations have been described, the majority of which affect the extracellular domain of the protein. In this case, we revealed a mutation in the ligand-binding domain of CTLA4 gene (13). There are several case reports of CTLA-4 mutations indicating an extended spectrum of clinical features most of which are associated with immune dysregulation and autoimmunities other than recurrent upper and lower respiratory tract infections. Shubert *et al.* reported different heterozygote mutations in CTLA-4 as the underlying cause of hypogammaglobulinemia, recurrent respiratory tract infections, autoimmune cytopenia, autoimmune enteropathy, and infiltrative lung disease in the affected members of the studied families. They also found the same mutations in healthy the members of these families. In their study, the age of disease onset ranged from 7 to 40 years; they further mentioned that healthy young carriers might later develop the disease (14). In our case, the first symptoms developed at four years of age, and other family members were not assessed afterwards. We suspect that the same mutation is the cause of the symptoms. Kuehn *et al.* identified a heterozygous mutation in patients with severe immune dysregulation and discovered the progressive loss of circulating B cell (6). As was determined in our case, B cell was lower than normal limits for his age, which was consistent with his humoral immunodeficiency. However, opportunistic infections are not a common feature in CTLA-4 deficiency. Our patient had two episodes of CMV colitis with severe diarrhea, from which he partially recovered after a long period of treatment. Hayakawa *et al.* reported a Japanese patient with autosomal dominant heterozygous single nucleotide insertion in CTLA-4 who develpoed gastric cancer. They reported CMV gastritis in their patient prior to gastric cancer diagnosis; they proposed this infection as a possible case of adenocarcinoma in their patient. This emphasizes the importance of an accurate screening of our patient for malignancy development (5). Treatment options for patients with CTLA-4 deficiency highlight two major characteristics of this disease: predisposition to 1) infection and 2) autoimmunities. As was done on our patient, immunoglobulin replacement therapy with or without antibiotic prophylaxis is suggested for patients with CTLA-4 deficiency's susceptibility to infection because any bacterial infection must be treated aggressively (13). Considering multiple autoimmunities in these patients, immunosuppressive compounds such as corticosteroids, sirolimus, rituximab, and anti-tumor necrosing factor drugs are usually required. Nevertheless, they may deteriorate the immunodeficiency status. A new proposed treatment is Abatacept (CTLA-4-Ig) which is a fusion protein comprised of the Fc region of the immunoglobulin IgG1 fused to the extracellular domain of CTLA-4. It selectively inhibits T cell activation through inhibiting costimulatory ligands such as CD80/CD86 (also known as B7-1 and B7-2, respectively). In other words, Abatacept prevents antigen-presenting cells (APCs) from delivering co-stimulatory signals. This treatment approach was approved for RA treatment in Europe in 2007. At first, it was recommended as a therapeutic option only in patients with inadequate response to tumor necrosis factor α (TNF-α) inhibitors; later, it was recommended as one of the first-line treatments for rheumatoid arthritis (15). Using Abatacept, Danieli MG *et al.* successfully treated severe enteropathy in a patient with a definitive diagnosis of CTLA4 deficiency. They described the efficacy and safety

of Abatacept in a patient with documented CTLA-4 deficiency, particularly in inducing and maintaining remission of enteropathy (11). The final therapeutic for resistant immune dysregulation is hematopoietic stem cell transplantation (HSCT) (16). Although various treatment options are available for autoimmunity manifestations of CTLA-4 deficiency, the treatment option is unclear. In conclusion, CTLA-4 is predominantly expressed on activated T-cells and regulatory T-cells; thus, CTLA4 deficiency can cause multiple autoimmunities. In this case report, we presented a patient with c.436G>A mutation in exon 2 of CTLA-4 gene, leading to missense variant p.G146R. One of the main characteristics found in this case was CMV-related colitis, but opportunistic infections were not frequent. Such patients are susceptible to different malignancies; therefore, they must be under close monitoring for possible complications; this becomes particularly important in cases such as ours who have a history of CMV infection. Although we implemented all the available treatments such as IVIG therapy, immunosuppressive drugs, and antibiotic therapy, we were not able to control the patient's diarrhea due to colitis, which remained a challenging issue. We recommend bone marrow transplantation as the most optimal therapeutic approach in this life-threatening condition.

CONSENT

We would like to appreciate our patient and his family for their adherence and our department staff for their support. Written informed consent was obtained from the patient`s legal guardians for publication of this case report and its accompanying information. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

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