

Case Report

A Case of CVID-Associated Inflammatory Bowel Disease with CTLA-4 Mutation Treated with Abatacept

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Received: 30 July 2019; **Accepted:** 14 August 2019; **Published:** 18 November 2019

Abstract

Background: CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) deficiency is a widely known cause of immunodeficiency and autoimmunity, determining an immune dysregulation syndrome. We here present the case of a Caucasian woman with common variable immunodeficiency (CVID) and severe enteropathy secondary to CTLA-4 deficiency, successfully treated with abatacept.

Case Report: A 54-year-old woman affected by CVID, treated with 20% subcutaneous immunoglobulin, came to our attention for chronic diarrhoea, severe malabsorption and significant weight loss, requiring parenteral nutrition. Her past clinical history comprised psoriatic arthritis, idiopathic thrombocytopenic purpura and multifactorial anemia. She started treatment with glucocorticoids and adalimumab, with partial benefit. However, after six months anti-TNF-alpha agent was withdrawn for recurrent serious infectious episodes. Due to the complex phenotype, we performed a genetic analysis, which revealed the presence of a CTLA-4 heterozygous mutation, with consequent CTLA-4 deficiency. We decided therefore to start abatacept, a CTLA-4 fusion protein, at the dose of 125 mg/week subcutaneously in association with budesonide, with improvement. At one year of follow-up diarrhea is still in remission and the patient has gained weight. Even cutaneous lesions improved, whereas we documented only a partial benefit for peripheral arthritis.

Conclusion: CVID could present with complicated phenotype and immune dysregulation, underlying a more complex syndrome, like CTLA-4 deficiency, thus suggesting the importance of genetic investigations in selected patients. Abatacept is a potential effective treatment in patients with documented CTLA-4 deficiency, in particular to induce and maintain remission of enteropathy.

Keywords: Abatacept; Common variable immunodeficiency; CTLA-4; Genetic; Precision Medicine; Next Generation Sequencing (NGS); Whole Exome Sequencing (WES)

1. Introduction

Common variable immunodeficiency (CVID) is the most common symptomatic primary immunodeficiency of the adulthood, characterized by low serum immunoglobulin levels and impaired specific antibody response to vaccines. Clinical manifestations comprise recurrent infections, autoimmune diseases, granulomatosis, gastro-intestinal involvement with malabsorption and increased susceptibility to cancer. CVID is usually a sporadic disorder; in a minority of cases, it is possible to recognize a monogenic defect, whereas in most patients a polygenic cause is probable. Genetic analysis thus became an important step in the care of patients with CVID [1-4]. Heterozygous mutations of the immune checkpoint protein CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) are increasingly reported in patients with CVID and concomitant autoimmunity, determining an immune dysregulation syndrome [5]. The recognition of this condition is important since it could offer new treatment options, such as abatacept, a CTLA-4-immunoglobulin fusion protein [6].

We describe the case of a 54-year-old Caucasian woman, with a previous diagnosis of CVID and a severe malabsorption syndrome due to immune mediated enteropathy, successfully treated with abatacept after genetic demonstration of CTLA-4 heterozygous mutation.

2. Case Report

The patient came to our attention on May 2011 when she was 46 years-old for recurrent upper respiratory tract infections with the finding of severe hypogammaglobulinemia of all immunoglobulin classes. Her complex past medical history included:

- In 1989, at the age of 24 years, immune thrombocytopenia (ITP), requiring splenectomy and prolonged treatment with glucocorticoids;
- In 1999, chronic diarrhea lasting since the age of 34 years;
- Cutaneous psoriasis associated with spondyloarthritis, diagnosed in 2000;
- Osteoporosis and femoral head necrosis linked to chronic steroid therapy (2002).

No familiar cases of immune disorders were reported. We excluded a secondary cause of hypogammaglobulinemia, whereas impaired B cell maturation and absent response to tetanus toxoid were demonstrated. According to ESID criteria, the patient obtained the diagnosis of CVID. She started replacement therapy with intravenous

Immunoglobulin (IVIg), switched to subcutaneous Immunoglobulin (SCIg, Hizentra® [CSL Behring King of Prussia, PA, USA], 8 g/weekly) because of poor venous access, with the achievement of protective serum IgG levels. Following replacement therapy, the number of respiratory infections rapidly reduced, whereas chronic diarrhea persisted and gradually got worsening, influencing a severe weight loss (the minimum weight recorded was 33 kg in 2016).

In 2011 and in the following years, blood tests were consistent with a malabsorption syndrome. We excluded an infective agent determining malabsorption: stool exams, blood tests and digestive tract biopsies resulted negative for the main gastrointestinal pathogens (including *G. lamblia*). We excluded Small Intestinal Bacterial Overgrowth (SIBO), pancreatic failure and neoplasm. As for celiac disease, gluten-free diet appeared to be effective, but just for a short period; the search for specific antibodies and HLA haplotypes was negative. We did not test the patient for lactose intolerance because her diet was already lactose-free. Gastroscopy revealed a mild gastroduodenitis, negative for *H. pylori*. Colonoscopy showed a chronic colitis, with ulcers and inflammatory pseudo-polyps. Histopathological examination revealed crypt distortion, crypt micro-abscesses, a mucosal inflammatory infiltrate composed by lymphocytes and neutrophils and some aspects resembling a collagenous colitis.

The final diagnosis was “COVID-associated inflammatory bowel disease”; she received a steroid course (starting with intravenous methylprednisolone 1.5 mg/kg/day with appropriate tapering) leading to a partial clinical response. In 2017 to induce and maintain remission, we introduced adalimumab at the dose of 40 mg subcutaneously (s.c.) every 2 weeks, which was also indicated for psoriatic arthritis. As replacement treatment, the patient continued 20%SCIg (8 g/weekly) and started a supplemental parenteral nutrition by a central venous catheter (CVC). Unfortunately, six months later several serious infective complications, including a CVC infection due to *S. aureus*, lead us to withdraw adalimumab.

In 2018, considering the severe comorbidities and the suboptimal therapeutic response to anti-TNF-alpha agent, we decided to asses a genetic test in order to find out an underlying genetic cause of immunodeficiency, once obtained informed consent from the patient. The exonic regions of the target genes were selected and enriched using Roche NimbleGen SeqCap EZ Choice technology and the genetic examination were performed via NGS (MiSeq Illumina) reaching 98X medium coverage level and 30x reading depth. Fifty-nine genes of interest were selected according to the pathology of the patient and the polymorphisms discovered were confirmed via Sanger sequencing. A total of two interesting polymorphisms were found:

- 1) A CTLA-4 (NM_005214.4) deficiency due to gene heterozygous substitution consisting in a transversion T>G in position 529 causing a Tyrosine (177) to Aspartate missense mutation. This non-polar to polar mutation falls in the exon number 3, implied in a transmembrane domain rich of non-polar amino acids (AAs). This substitution has been described in association with a clinical picture of immune mediated thrombocytopenia, autoimmune neutropenia and lymphoid reactive hyperplasia [7]. The clinical situation of our patient was compatible with the phenotype associated with the *CTLA-4* gene mutation.

- 2) A de-novo heterozygous *FCGR3A* (NM_001127593.1) single nucleotide polymorphism (SNP) was discovered, a transversion A>T in position 694 causing an Asparagine (232) to Tyrosine substitution, falling in exon number 6. This SNP is reported as *Minor Frequency Allele* (MAF) in dbSNP (rs155866423). His biological significance remains uncertain and this mutation is not known to be implied in pathological situations. Other mutations involving *FCGR3A* are present in neutropenia following rituximab treatment in patients with rheumatic disease [8].

According to the data present in literature, we decided to administer her abatacept at the dose of 125 mg/week s.c. associated with an oral topical steroid (budesonide 9 mg/day). The patients started this schedule after the removal of CVC and the consequent interruption of parenteral nutrition, in order to avoid other infectious complications. Despite the absence of parenteral support, at one year follow-up the patient has gained weight (36 kg) and diarrhea was in remission. However, the attempt to reduce budesonide dose under 6 mg/day was unsuccessful, because of the rapid onset of severe iron deficiency anemia connected to subclinical gastrointestinal bleeding. Abatacept was effective even on cutaneous psoriasis. However, she had only limited beneficial on her right wrist arthritis, requiring the introduction of methotrexate 10 mg/week s.c., with initial clinical improvement. The patient is on close follow-up.

3. Discussion

Gastrointestinal involvement (GI) in CVID is common, ranging from 20 to 60% of cases. We can identify four different groups of GI manifestations, linked to infection, inflammation, malignancy and autoimmune disorders. In our patient, non-infectious diarrhea was a consequence of an inflammatory bowel disease, leading to chronic diarrhea, weight loss and malabsorption.

The treatment of CVID-associated enteropathy, including glucocorticoids and immunosuppressive agents, can be challenging, as the use of immunosuppressants can increase the infection risk. Even if a supplemental parenteral nutrition is useful, the use of CVC could amplify the probability of sepsis. Usually, in CVID-associated enteropathy, steroids can be used as monotherapy or in association with conventional disease-modifying anti-rheumatic drugs or biological ones such as anti-TNF-alpha agents. Chua et al. demonstrated a useful role for infliximab therapy in three cases of CVID patients with severe enteropathy resistant to steroid treatment [9]. Even though more data are necessary, further evidences documented the emerging role for anti-TNF-alpha agents in patients with CVID-associated enteropathy, particularly in those with refractory forms and in those who necessitate of steroid sparing [10]. Our patient preferred adalimumab due to ease of administration. Unfortunately, severe complications lead us to withdraw this treatment and to test other therapeutic options.

In our patient, the genetic analysis revealed a heterozygous mutation, linked to CTLA-4, thus providing a new therapeutic option. CTLA-4, also known as CD152, is a receptor expressed in activated T-cells that acts as an immune checkpoint, down-regulating immune response. CTLA-4 demonstrated both extrinsic and intrinsic activity. The extrinsic activity explains his nature of cellular receptor and is essential for regulatory T cells (Treg) effective

function [5]. However, also CTLA-4 internalization and endocytosis has been confirmed, suggesting a pivotal role in its activity and correct regulation by interactions with the plasma membrane. Thus, mutations related to transmembrane domain can alter protein localization and stabilization in the plasma membrane, causing a CTLA-4 deficiency. Due to plasma membrane high hydrophobic nature, transmembrane domains (composed of a series of α -Helix) have to maintain this characteristic to be correctly internalized and to perform their function. Exon number 3 has been documented to be implied in a transmembrane domain and Tyr in position 177, a non-polar AA, falls in an α -helix secondary structure characterized by the presence of many non-polar AAs. The substitution of Tyr177 with an Asp, a polar charged AA, will modify the charge and thus the stability of this α -Helix causing problems in localization and function of CTLA-4 [11-13]. In this regard, CTLA-4 is homologous to the T-cell co-stimulatory protein CD28, with opposite functions in T-cell activation [5]. T-cell activation is a complex process that requires several signals and the interactions between CD28 stimulatory signal and CTLA-4 inhibitory signal are essential to regulate the activity of T-cells. CTLA-4 is also constitutively expressed in Treg, leading to an inhibition of T-cell proliferation and reduced effector functions [14]. By controlling self-reactive and tumor-reactive T cells, CTLA-4 may modulate the immune tolerance. Thus, in patients with impaired CTLA-4 function, autoimmune manifestations may arise.

Therapy targeting CTLA-4 is based on the administration of abatacept, a human fusion protein that modulates T-cells activity, restoring the insufficient CTLA-4 activity. The pathophysiology of autoimmune enteropathies is probably explained by a hyperactive immune state in the setting of a T-cell regulatory defect [15]. The destruction of epithelial cells is caused by the activation of CD4 T lymphocytes upon exposure of the epithelial cell surface of enterocytes. Gupta et al. have shown that abatacept can be a useful therapeutic strategy in autoimmune enteropathies (non CVID-related) refractory to conventional therapies [15].

In 2015, Lo et al. were the first to describe the rationale about the use of abatacept in diseases involving CTLA-4 pathway [16]. They reported the promising results following abatacept use in patients with LPS-responsive beige-like anchor (LRBA) mutation, a cytosolic protein that regulates CTLA-4 expression. Eren Akarcan et al. have described the cases of two brothers with a novel mutation of *LRBA* [16]. At clinical presentation, the younger brother had chronic diarrhoea, the elder one autoimmune haemolytic anaemia. Both developed hypogammaglobulinemia, enteropathy and lung involvement. For a target-specific therapy, the brothers received abatacept in addition to corticosteroids and in the elder to mycophenolate mofetil. The older brother achieved a satisfactory respiratory improvement in a few months, while the younger developed fungal oesophagitis, and pneumonia after the first dose of abatacept. In this last case, abatacept was stopped for fungal infections probably related to immunosuppressive therapy. These results showed the possibility to treat LRBA deficiency with abatacept in associations to corticosteroids and immunosuppressants drugs even if the available data are still limited.

4. Conclusion

Our case is related to a woman with a complex immune dysregulation syndrome linked to a genetic defect of CTLA-4. Her clinical picture comprises immune mediated thrombocytopenia, antibody deficiency, severe enteropathy and

skin and joint involvement due to psoriasis. According to our experience in this patient, abatacept was useful in resolving gastrointestinal symptoms in association with topical steroid therapy and in improving cutaneous psoriasis. Unfortunately, it was only partially effective on arthritis.

In the workup of patients with CVID, genetic analysis has now gained increasing importance. While CVID caused by monogenic mutation accounts for less than 20% of all cases, cumulative effects deriving from polygenic determinants are more frequently involved in CVID pathogenesis. These polymorphisms include both coding regions and non-coding regions mutations, which could be gene-gene interactions, regulatory variations in non-coding regions involving transcription and translation rates, mRNA nuclear retention, regulatory RNAs (e.g. miRNA) and alternative splicing [17]. Thus, focusing analysis only over exonic regions of target genes will create biases and will not allow discovering real causes of polygenic CVID, a pivotal aspect needed for the evolution of personal-medicine treatments. Moreover, Whole Exome Sequencing (WES) or Target PID (Primary Immunodeficiencies) panel will identify only 60% of cases, due to incomplete covering of exons by WES, so these techniques could not find a mutation in these regions, hence not potentially diagnosing a PID [18]. Not less important, cellular markers in CVID patients can vary even in patients with identified genetic defects: altered methylation of CpG sites in critical B cells genes were observed, suggesting environmental implications that modify disease susceptibility and molecular phenotype [19]. Whole Genome Sequencing (WGS) combined with RNAseq discovered an average of 9.4 variants per patient and 84% of variants were shared among two or more patients [20]. Thus, due to the heterogeneous complex of causes, NGS has established a great importance in PID patient care. WGS combined with transcriptome analysis, methylation patterns, regulator RNA, proteomic and metabolomic studies may provide a molecular diagnosis in previously unclassified cases and lead to identify new therapeutic options or to provide a prognosis or risk-group stratification.

Understanding the genetic variability in CVID is critical to develop personalized approaches to treatment, comorbidity monitoring and care of patients with CVID. Besides coding region defects, non-coding alterations, regulatory RNAs alteration, and epigenetic variations including environmental factors can influence the clinical and molecular phenotype and have an impact on the full-blown expression of CVID. Primary immunodeficiencies (PID) are a group of rare diseases, which will greatly benefit from NGS, with a massive increase in the causative genes identified in the past few years. The fast development of NGS techniques has accelerated the discovery of novel PIDs drastically, as exemplified by more than 300 genetically defined single gene PIDs to date. In addition to WGS techniques, RNAseq, methylation and acetylation profiles, ChIP-seq and several biomolecular techniques are now available and will permit to study and elucidate the molecular pathway involved in PIDs. Studies of epigenetics, proteomics and possibly metabolomics in combination could help to better define which pathways are dysregulated and open to therapeutic. This is a big step forward in implementing genomic medicine as part of tailored care of individual patients suffering from common and rare diseases.

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Citation: Danieli MG, Pedini V, Menghini D, Mezzanotte C, Verga JU, Mannurita SC, Gambineri E. A Case of CVID-Associated Inflammatory Bowel Disease with CTLA-4 Mutation Treated with Abatacept. *Archives of Clinical and Medical Case Reports* 3 (2019): 481-488.



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