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LETTER TO EDITOR

Treatment of Intracerebral Lesions with Abatacept in a CTLA4-Haploinsufficient Patient

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To the editor,

In a subgroup of patients with common variable immune deficiency (CVID), the underlying genetic defect is a heterozygous mutation in the *CTLA4* gene [1, 2]. CTLA-4 is a negative regulator of T cell responses whereby T cell activation is balanced by binding of two opposing receptors, CD28 (stimulatory) and CTLA4 (inhibitory), to CD80 and CD86 expressed on antigen-presenting cells. CTLA-4 is constitutively expressed on $CD4^+$ regulatory T cells (Treg), whereas on conventional $CD4^+$ T cells, its expression is induced upon activation. In mice, deficiency of CTLA-4 leads to extensive auto-immunity due to an increase in auto-reactive T cells [3]. In addition to the increased incidence of infections caused by impaired antibody responses, CTLA-4 deficiency in man is also associated with auto-immune diseases. Their response to treatment is variable and prolonged high-dose steroids are generally needed [1, 2]. A new promising targeted approach is treatment with abatacept, which is a fusion protein (human IgG1 coupled to the extracellular domain of CTLA-4) that can potentially restore immune dysregulation. However, published experience of abatacept treatment in patients with CTLA-4 deficiency is limited [4, 5].

Patient Description

Our patient is an adult man who was diagnosed with CVID at 20 years of age (in 2003), when he presented with several streptococcal-related pneumonias, auto-immune hemolytic ((Hb 2.5 mmol/L) IgG Coombs positive) anemia, and hypogammaglobulinemia (IgG/A/M, see Supplementary Table 1). In the months after diagnosis, a thrombocytopenia (150×10^9 cells/mL) with a splenomegaly on abdominal ultrasound became evident. In the absence of measurable auto-antibodies, the thrombocytopenia might be caused by cellular auto-immunity or/and hypersplenism. Vaccination responses were not tested and CVID was diagnosed based on mentioned symptoms and findings. Soon after this diagnosis, treatment was initiated with intravenous immunoglobulin replacement (60 g each 3 weeks) which led to a decrease in pneumococcal infections. The auto-immune hemolytic anemia was treated with steroids with a good response (Hb 9.2 mmol/L). Four years later, he developed generalized epileptic seizures. Cerebral magnetic resonance imaging (MRI) showed several lesions in the white matter located in the right hemisphere and cerebellum (Fig. 1a). A stereotactic brain tissue biopsy taken from the lesion in the right temporal lobe showed a chronic inflammatory infiltrate to a large extent consisting of $CD4^+$ and $CD8^+$ T cells with some areas of granuloma formation (Fig. 1b). Cerebral spinal fluid analysis showed low cell counts (26 cells/uL), no malignant cells, and was negative in gram staining, culture and PCR for JCV virus, enterovirus, and human parechovirus. Importantly, T cell receptor repertoire analysis showed a clear polyclonal T cell receptor repertoire excluding a T cell lymphoma. Staining for microorganisms and fungi (PAS-D, Gram staining, and Giemsa and Grocott) and for CMV, varicella zoster, herpes simplex, BK-virus, and EBER were all negative. Taken together, the

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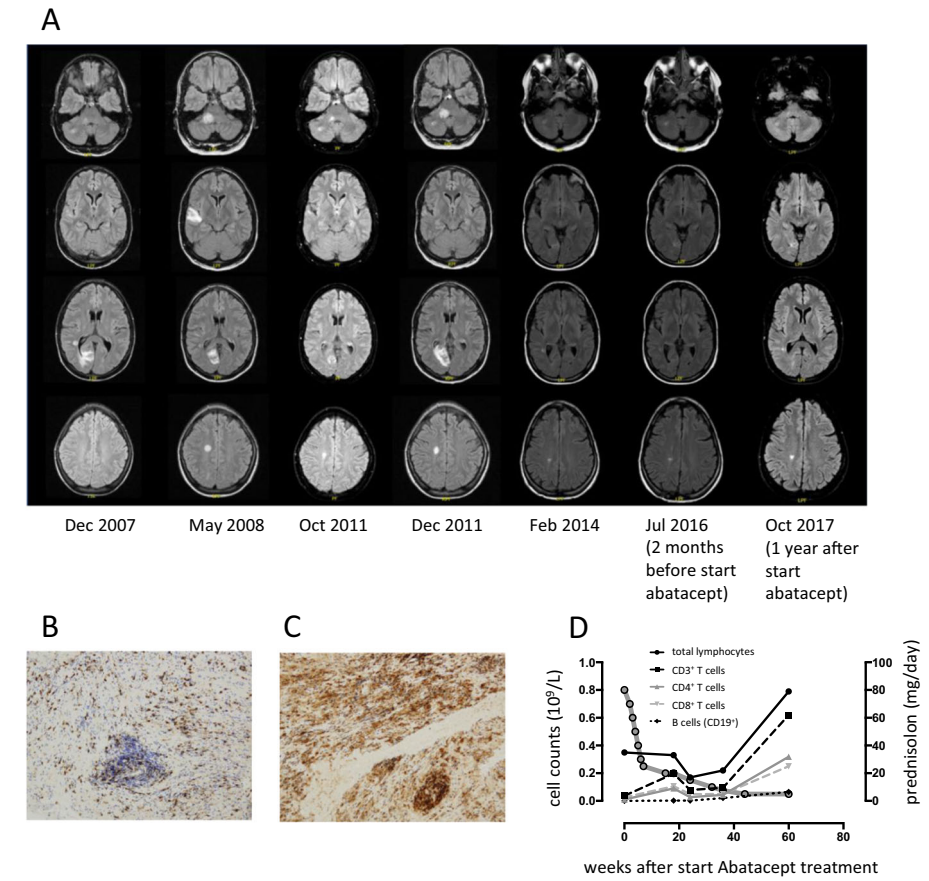
A
Dec 2007
May 2008
Oct 2011
Dec 2011
Feb 2014
Jul 2016 (2 months before start abatacept)
Oct 2017 (1 year after start abatacept)
B
C
D
total lymphocytes
CD3+ T cells
CD4+ T cells
CD8+ T cells
B cells (CD19+)
cell counts (10^9/L)
prednisolon (mg/day)
weeks after start Abatacept treatment
E
Gated on Total CD4+
Gated on CD25+FOXP3+Helios+
MFI:
1615
1727
1797
1931
143
3861
4448
3651
3391
143
After Abatacept
Before Abatacept
Control 2
Control 1
Unstained
CTLA-4
F
CD4+CD25+FOXP3+ (%)
CD4+CD25+CD127- (%)
CD4+FOXP3+Helios+ (%)
Healthy Controls
Before Abatacept
After Abatacept

◀ **Fig. 1** **a** Sequential axial FLAIR brain MRI scans showing the hyperintense lesions in the cerebellum, pons, occipital and temporal lobes, and semioval center over time. **b**, **c** Histopathology of biopsy from right temporal lesion showing parenchymal and perivascular T cell infiltrates (**b** CD4 staining; **c** CD8 staining) (The biopsy was obtained in July 2008). **d** Overview of lymphocyte counts over time since start of abatacept. Week 0 is a week just prior to start abatacept. Left Y-axis shows lymphocyte counts; the right Y-axis shows the daily dosage of prednisolone (mg) (open circles). **e** Histograms showing the intracellular and cell surface expression of CTLA-4 on total CD4 T cells and Tregs directly ex vivo. **f** Graphs showing the quantification of Treg numbers as defined in Supplementary Fig. 1

pathological diagnosis was a polyclonal T lymphocytic inflammatory infiltrate.

He was started on high-dose steroids (80 mg prednisolone/day) in combination with prophylactic cotrimoxazol (960 mg OD) and valaciclovir (500 mg TD). Several second-line courses of immunosuppressive drugs were added attempting to lower the steroid dose (rituximab, methotrexate, and cyclophosphamide). However, tapering of steroids was still not possible as even lowering the dose with 10 mg/day already led to the development of new neurologic deficits (peripheral facial nerve palsy and limb weakness) with progression of size and number of intracerebral lesions on cerebral MRI (Fig. 1a). Therefore, the patient continued to receive high-dose steroids (80 mg/day) for 8 years and remained free of neurological symptoms under this regimen. Unfortunately, 4 years after start of prednisolone treatment, he developed a disseminated atypical mycobacterial infection (*Mycobacterium avium* (*MAC*)) localized in gut, spleen, and bone marrow (PCR and culture positive) for which anti-mycobacterial treatment (rifampicin, clarithromycin, and ethambutol) was initiated. Just before referral to our hospital, whole genome sequencing was performed (Prof Hammarstrom, Karolinska Institute) that revealed a novel heterozygous mutation in the *CTLA4* gene located in the ligand binding domain (stopgain exon 2 c.T450G:p.Y150X). Following this new insight in the underlying cause of the patient's CVID, we started treatment with abatacept (750 mg every 4 weeks) to replace the lacking CTLA-4-associated immune regulation. Concomitantly, prednisolone was tapered as shown in Fig. 1d, until a dosage of 5 mg OD. The patient did not experience relapse of any neurological symptoms nor other auto-immune-related disease manifestations and repeated MRI showed no new lesions (Fig. 1a). Furthermore, repeated bone marrow examination, 10 months after starting abatacept and under 7.5 mg prednisolone, showed no growth of MAC, but still PCR positivity. Also, in blood cultures, no MAC growth was detected, both suggestive of partial control of the MAC infection. Over the period since start of abatacept, IVIG was continued.

To evaluate the effect of abatacept on the patient's immune system, characterization of lymphocyte subsets and CTLA-4 expression was performed before and during treatment. Just before start of abatacept, the patient's peripheral blood counts showed a microcytic anemia suggestive of chronic disease (low total serum iron, high serum ferritin, low/normal total iron binding capacity), thrombocytopenia, and a profound $CD4^+$ and $CD8^+$ T and B cell lymphopenia. In the first period after start of treatment, T and B cell counts remained low, but they remarkably recovered 14 months after initiation of abatacept. Also, the anemia recovered with a near normal Hb value (Hb 7.4 mmol/L) (supplemental Table). Thrombocyte counts remained low, potentially due to pre-existent splenomegaly. The expression of CTLA-4 on total $CD4^+$ T cells as well as on Tregs (defined as $CD4^+FOXP3^+Helios^+$) both before and after start with abatacept was comparable to healthy controls. However, Treg cell counts and expression of different Treg markers such as Helios, CD25, and FOXP3 were dramatically reduced before treatment and recovered during abatacept treatment. (Supplementary Table 1 and supplementary Fig. 1).

Discussion

A subgroup of patients with a CVID-like phenotype suffer from severe auto-inflammatory intracerebral manifestations. In a proportion of these patients, this immune dysregulation can be attributed to defects in CTLA-4 itself or in LPS-responsive beige-like anchor protein (LRBA) that regulates trafficking of CTLA-4 intracellularly. In patients with a profound auto-inflammatory clinical signature, defects in either CTLA or LRBA should be considered, because in both patient groups, abatacept treatment can provide a successful therapeutic strategy. Here, we describe a patient with severe intracerebral T cell-mediated inflammation that was refractory to treatment with conventional immunosuppressive drugs, who was successfully treated with abatacept allowing steroid tapering. In the period of treatment (now over a year), we did not observe toxic side effects, neither treatment-related infectious complications. The clinical course with stop in further neurological symptoms and new CNS lesions upon substantial reduction of steroid dose combined with abatacept treatment may suggest that abatacept restores immune dysregulation related to CNS disease in this host.

Our patient had very low to undetectable B cell and $CD4^+$ and $CD8^+$ T cell counts before start of abatacept that finally recovered at about 14 months of treatment with this CTLA4-fusion protein, concomitant with tapering of steroids. Whereas the mechanism behind T lymphopenia is unclear in CTLA-4 deficient patients, the reduced B cell count is assumed to be due to a pro-apoptotic tendency. The lymphocyte recovery in our patient might be due to both steroid tapering and restored immune regulation by abatacept. Despite the heterozygous CTLA-4 mutation, we observed a normal CTLA-4 expression on

total $CD4^+$ and Tregs. This observation leaves the possibility that this new mutation in the ligand binding domain may affect ligand binding. The question is to which extent Treg function is modulated by abatacept. In our patient, upon start of treatment, expression of CD25 and FOXP3 increased. Given the central role of FOXP3 in Treg regulation, this increased expression may be an indirect reflection of restoration of Treg function. However, due to the low T cell counts, we were unfortunately not able to functionally investigate Treg function in vitro.

Finally, at the time the patient developed the systemic mycobacterium avium infection, he had been on high-dose steroids (around 80 mg day) for 4 consecutive years and had low $CD4^+$. We would argue that both steroid treatment and these low CD4 T counts have contributed to the susceptibility to the mycobacterium avium infection. This assumption is further supported by the signs of control of infection under tapering steroids and recovery of CD4 T cell counts.

Taken together, our case report illustrates the beneficial effect of abatacept in the treatment of severe treatment-refractory inflammatory manifestations in CTLA-4-related CVID and underscores the value of (early) genetic diagnostics that may guide targeted therapies.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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