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B cell depletion can be effective in multiple sclerosis but failed in a patient with advanced childhood cerebral X-linked adrenoleukodystrophy

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***Abstract*:** Rituximab exerts its clinical efficacy by its specific pattern of depletion of CD20+ B lymphocytes and it has been demonstrated that rituximab is an effective treatment for relapsing remitting multiple sclerosis. X-linked adrenoleukodystrophy (X-ALD), the most common monogenetic neuroinflammatory disorder, shares substantial overlap with multiple sclerosis in the neuropathological changes found in brain tissues in advanced stages of the disease. While there is no effective therapy for these patients, we hypothesized that rituximab might be effective in arresting the neuroinflammatory process. Our detailed clinical, imaging and immunological data revealed that rituximab is not effective in advanced stages of X-ALD and consequently should not be applied for compassionate use in these patients.

Keywords: B cells, multiple sclerosis, rituximab, X-ALD, X-linked adrenoleukodystrophy

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Introduction

X-linked adrenoleukodystrophy (X-ALD) is the most common monogenetic neuroinflammatory disorder affecting the central and peripheral nervous system as well as adrenal cortex and testis.[1] The disease is caused by mutations in the *ABCD1* gene encoding the adrenoleukodystrophy protein (ALDP), a peroxisomal ATP binding cassette (ABC) transporter, resulting in the accumulation of very long-chain fatty acids (VLCFA) in organs and plasma.[1] Phenotypes range from asymptomatic carriers to severe childhood cerebral X-ALD (CCALD) with inflammatory demyelination in the central nervous system (CNS).[1] So far, hematopoietic stem-cell transplantation (HSCT) and hematopoietic stem-cell gene therapy are the only treatment options, but are restricted to patients without advanced neurologic deficits.[2–6] The inflammatory nature of the lesions with the presence of lymphocytes, and the significant blood brain barrier damage, has led to the use of various immunomodulatory or immunosuppressive treatments, such as steroids, cyclophosphamide, immunoglobulins and interferons in single patients; however, these have mostly been unsuccessful.[7–10]

Case report

This boy is the first son of German parents, a healthy father and a mother with mild symptoms of adrenomyeloneuropathy (AMN). Pregnancy was complicated by gestational diabetes; delivery was *via* Caesarian section due to HELLP syndrome in the 36th week of gestation. Early development was normal; he was speaking his first words at 6 months, and walking at 14 months. At the age of 2 years his skin was darker when compared with that of his younger siblings, pointing to an adrenal insufficiency that was not diagnosed at that time. By the age of 4 years, he had suffered from three severe episodes of gastroenteritis with lethargy and insatiable vomiting. From the age of 7 years, his learning and working patterns at school slowed down. School phobia was assumed and led to a change of school;

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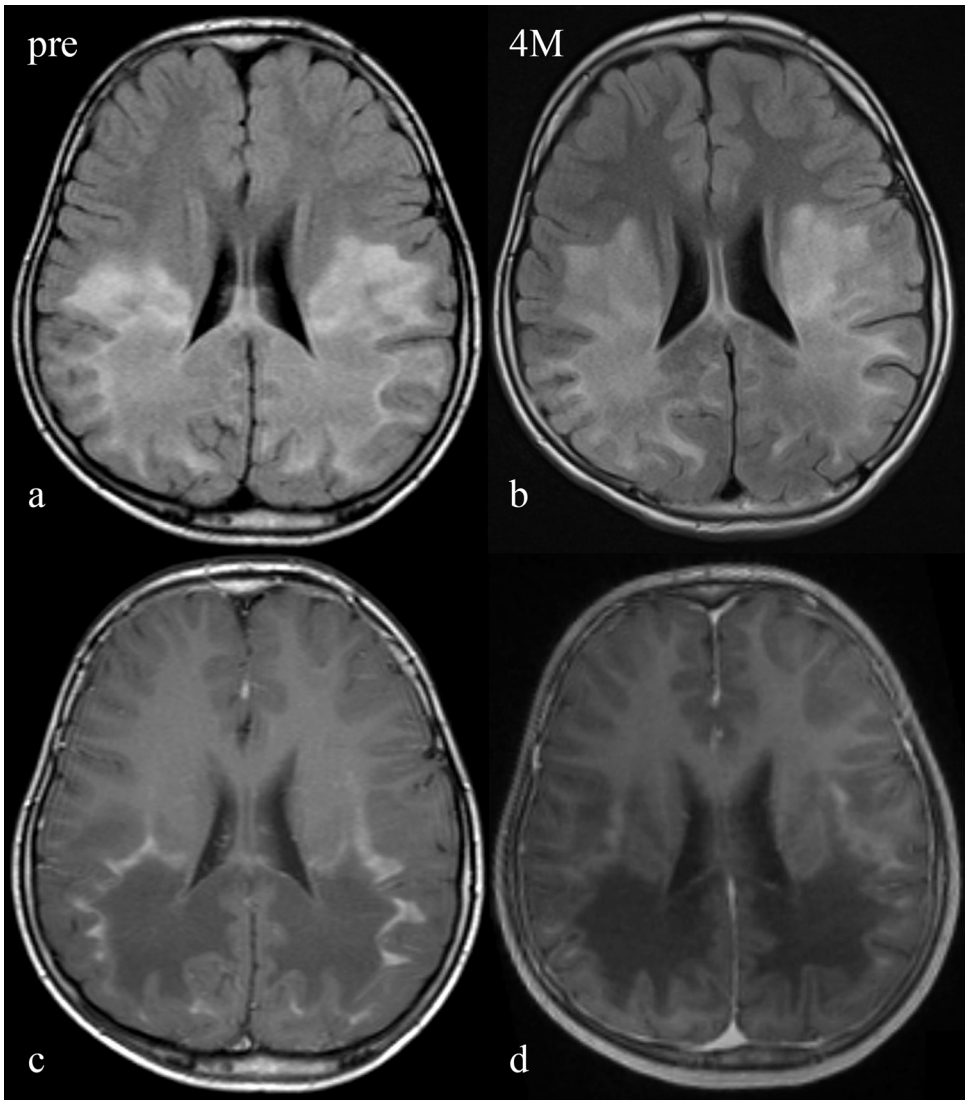
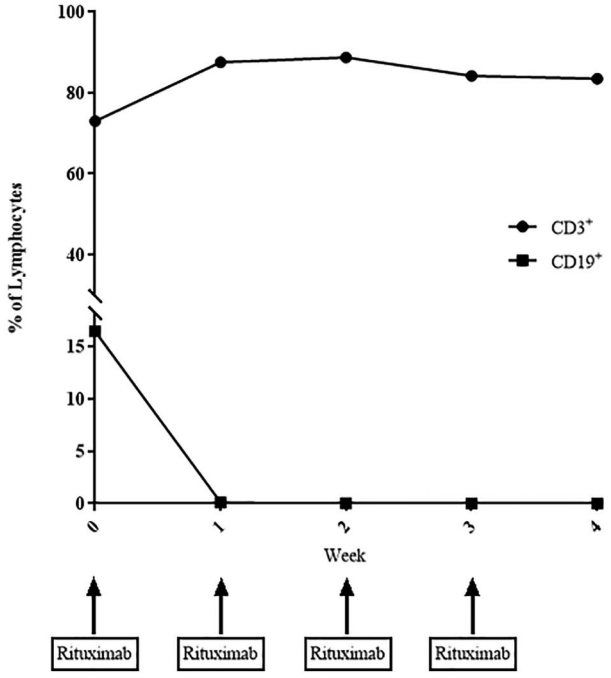


Figure 1. Magnetic resonance imaging (MRI) data. (a) Initial Cerebral MRI (cMRI) before rituximab treatment: symmetric hyperintense T2 FLAIR signal in the parieto-occipital white matter (age 7 years, 10 months; Philips 1.5T T2 FLAIR axial). (b) Last cMRI 4 months after rituximab treatment: symmetric hyperintense T2 FLAIR signal in the parieto-occipital white matter with progression of inflammatory demyelination extending to the frontal lobe (age 8 years, 3 months; SIEMENS 1.5T T2 FLAIR axial). (c) Initial cMRI before rituximab treatment: symmetric hypointense T1 signal in the parieto-occipital region with contrast enhancement at the advancing margin (age 7 years, 10 months; Philips 1.5T T1 post gadolinium axial). (d) Last cMRI 4 months after rituximab treatment: symmetric hypointense T1 signal in the parieto-occipital region with contrast enhancement at the advancing margin extending to the frontal lobe (age 8 years, 3 months; SIEMENS 1.5T T1 post gadolinium axial).

6 months later, reading difficulties and amnestic aphasia became overt. Neuropsychological tests were performed, with striking pathologic results in visual and work performance. Cerebral magnetic resonance imaging (cMRI) at age 7 years and 11 months revealed the classical X-ALD disease pattern with symmetrical hyperintense white matter lesions in bilateral occipital, parietal and temporo-dorsal regions as well as the splenium of the corpus callosum. Also, the posterior white matter abnormalities showed a linear contrast enhancement at the advancing margin in post-contrast T1-weighted images (Figure 1a–c). The commonly used assessment in patients with CCALD to evaluate the extent of lesions on MRI is the Loes score, with a value < 1 reflecting mild,



PBMCs	% CD3+	% CD19+
pre 1st Rituximab infusion	73	16,42
pre 2nd Rituximab infusion	87,52	0,12
pre 3rd Rituximab infusion	88,72	0,04
pre 4th Rituximab infusion	84,2	0,02
1 week after 4 th rituximab infusion	83,46	0

Figure 2. Analysis of peripheral immune cell subtypes: peripheral blood immune cell subtypes including CD19+ B cells and CD3+ T cells were evaluated prior to the first, second, third and fourth as well as after the fourth rituximab infusion. Normal percentages of CD19+ B cells and CD3+ T before the first rituximab infusion. CD19+ B cells decreased markedly after the first rituximab cycle, with further reduction to zero after the fourth rituximab infusion.

3–6 reflecting moderate and ⩾7 reflecting severe cerebral involvement.[11,12] Applying this scoring system to the first MRI (Figure 1a–c) results in a Loes score of 18.

Diagnosis of X-ALD was assumed, and confirmed by elevated plasma concentrations of VLCFA. At that time, the patient had impaired walking and direction difficulties. Retrospectively, the clinical symptoms pointing to adrenal insufficiency at age 2 years should have caused immediate diagnosis of X-ALD with MRI screening on a semi-annual basis to offer HSCT at the earliest time point of cerebral involvement. The disturbed learning and working patterns at age 6 years, 1 year before the admission to our hospital and confirmation of CCALD, mark the clinical start of cerebral involvement. In CCALD, inflammatory demyelination in the CNS can either predate cerebral symptoms or appear to develop at the same time as patients become symptomatic.[11,13,14] The combination of the clinical course and a Loes score of 18 in the first cerebral MRI provide evidence for an advanced stage of the disease with first inflammatory demyelination of the CNS at least 1 year before the diagnosis of CCALD and a typical rapid progression. Because of the clinical and neuroradiological CCALD advanced disease stage, HSCT was inapplicable.[15]

At age 8 years, after standard preliminary investigations for the treatment with the genetically engineered chimeric monoclonal anti-CD20 antibody rituximab, and written informed consent from the parents, the patient received 375 mg rituximab/body surface area at day 0, 7, 14 and 21 i.v. under standard conditions as compassionate use. No serious, limited or mild-to-moderate adverse events were noted. After the second infusion of rituximab, total depletion of B cells in the peripheral blood B-cell counts was demonstrated with fluorescence-activated cell sorting (FACS) analysis (Figure 2).

Despite this treatment, the clinical course of the patient was rapidly progressive, showing deterioration in motor and cognitive functions. cMRIs performed 2 and 4 months after the first rituximab treatment displayed progressive inflammatory

demyelination extending to the frontal lobe (Figure 1b+d). The patient died 18 months after the initial diagnosis of X-ALD and 6 months after the initiation of rituximab therapy. A written informed consent to publish the medical data and images was obtained from the patient's parents.

Discussion

For patients with advanced stage CCALD there are still no treatment options that slow down or halt the rapid neurological decline caused by a severe inflammatory demyelination. Neuropathological studies as well as the cMRI pattern in CCALD suggest immune-mediated mechanisms at least as part of the pathogenesis. In addition, biopsy and postmortem CCALD histopathology has overlapping features with multiple sclerosis (MS)[16] concerning the composition of the inflammatory infiltrate, although both diseases, MS and CCALD have a completely different pathogenesis. Whereas MS is clearly a peripherally driven autoimmune disease resulting in inflammatory CNS demyelination, inflammation in CCALD is most likely secondary to deposition of VLCFA or metabolic changes in oligodendrocytes. Therapeutic trials for MS have shown that anti-CD20, B cell depleting monoclonal antibodies like rituximab and ocrelizumab are highly effective in relapsing remitting MS.[17] For primary progressive MS, a recent trial with ocrelizumab (ORATORIO), has shown some effects on disability progression.[18,19] Therefore, we applied rituximab in this CCALD patient as an individual treatment approach. While rituximab was well tolerated and effectively depleted B cells in his blood, the extent of inflammatory CNS demyelination increased and his neurological condition further declined. As rituximab displays high efficacy in relapsing remitting MS, and less in primary or secondary progressive MS, one could propose that rituximab treatment earlier in the disease course might have been beneficial to the patient. As HSCT is an approved treatment option for patients in the early stage of cerebral involvement in CCALD, rituximab is not a treatment option.

This case demonstrates that a therapy with rituximab in advanced stage CCALD may not be effective despite proven B cell depletion. This observation shows that the role of B cells in the pathogenesis of inflammatory demyelination is different in X-ALD and MS. This suggests that peripheral B cells are not required to sustain the inflammatory CNS process in X-ALD, at least when the disease has already progressed significantly. Our observation is supported by two recent studies. The first demonstrated an early activation of two endoplasmatic stress sensors (PERK and ATF6 pathway) activating unfolded protein response (UPR),[20] and the second showed an impaired plasticity of macrophages as part of the pathogenesis of the acute inflammatory demyelination in CCALD.[21]

In conclusion, the authors do not recommend rituximab for compassionate use in advanced stage CCALD.

Author contributions

H. Rosewich: Study concept, acquisition of data, analysis and interpretation of data, drafting and revising the manuscript.

S. Nessler: Analysis and interpretation of data, drafting and revising the manuscript.

W. Brück: Analysis and interpretation of data, drafting and revising the manuscript.

J. Gärtner: Study concept, acquisition of data, analysis and interpretation of data, drafting and revising the manuscript.

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Conflict of interest statement

H. Rosewich and S. Nessler report no conflict of interest. W. Brück has received honoraria for lectures by Bayer Vital, Biogen, Merck Serono, Teva, Genzyme, Roche and Novartis. He is a member of scientific advisory boards for Teva, Biogen, Novartis and Genzyme, and receives research support from Teva, Biogen, Genzyme and Novartis. J. Gärtner has received honoraria for lectures and consultancy fees from Bayer, Teva and Novartis and research support from Novartis.

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