Tocilizumab for treating refractory haemolytic anaemia in a patient with systemic lupus erythematosus

SIR, Treatment of SLE is based on a judicious combination of glucocorticoids (GCs) and immunosuppressants. The variety of effective immunosuppressants has increased in recent years, but there is still a percentage of patients with resistant manifestations. Rituximab has been the first biologic drug effective in the treatment of resistant SLE [1–4]. Belimumab appears effective in SLE, but has not been tested in this context. We report a patient with SLE and autoimmune haemolytic anaemia (AIHA) resistant to GC and immunosuppressants treated with tocilizumab, a humanized mAb that blocks the IL-6 receptor.

A 52-year-old woman with SLE had AIHA resistant to GC and immunoglobulins and was treated with rituximab, four weekly doses of 375 mg/m², associated with CYC (two doses of 750 mg i.v. biweekly) and GC (deflazacort 60 mg/day, equivalent to prednison 50 mg/day, with posterior tapering). Complete remission was achieved. AIHA recurred 1 month after stopping GC, with new remission after another cycle of rituximab, and again 3 years later. A third cycle of rituximab was incompletely administered because of infusional reaction, without achieving remission. Deflazacort was increased to 90 mg/day (equivalent to prednisone 75 mg/day) and ciclosporin (4 mg/kg/day) was started. AIHA remitted, but recurred after stopping GC. Ciclosporin was replaced by MMF (2 g/day). After transitory remission, AIHA recurred 9 months later without response to GC (deflazacort 60 mg/day) and CYC (750 mg i.v.). Blood tests showed the following data: haemoglobin 57 g/l, reticulocytes 334.7 × 10⁹/l (15.8%), positive direct Coombs test, absence of schistocytes in blood smear, haptoglobin 20.9 mg/dl (normal range 30–200), lactate dehydrogenase 264 UI/l (normal range 135–225), total bilirubin 1.92 mg/dl (normal range 0.1–1.2), direct bilirubin 0.55 (normal range 0–0.35), CRP 11.1 mg/l, hypocomplementaemia and normal levels of platelets, vitamin B12, folic acid, iron and ferritin. She was treated with five daily pulses of methylprednisolone (1 g/day); doses of deflazacort and MMF were maintained and tocilizumab (8 mg/kg biweekly) was started after patient’s informed consent and approval by the Hospital Commission of Pharmacy were obtained. Four weeks later the haemoglobin level became normal and the other altered parameters improved (Fig. 1). GC was tapered and stopped 10 weeks after starting tocilizumab, which was reduced to 4 mg/kg biweekly at week 12. Compensated haemolysis data were detected at week 16, but rapidly improved after adding GC (deflazacort 30 mg/day) and increasing the dose of MMF (3 g/day) and tocilizumab (8 mg/kg biweekly). After achieving stabilization of blood disorders, we will try again to reduce the daily dose of deflazacort.

IL-6 is a pleiotropic cytokine produced by various cell types, with broad biological activities. It is expressed at high levels in inflammatory foci and seems to play a central role in chronic inflammation. There are data supporting the importance of IL-6 in the pathogenesis of SLE [5]. Blockade of IL-6 with tocilizumab is effective in RA, JIA and Castleman’s disease, and was also effective in a pilot open trial in a small group of patients with moderately active SLE [5]. Disease activity showed a significant improvement, with particularly favourable response of arthritis, and levels of inflammatory reactants, IgG, anti-dsDNA antibodies and plasma cells in peripheral blood were significantly reduced. There were no infusional reactions. Sixteen infections were detected in 11 patients, mostly mild and all with a good outcome. There was a dose-dependent decrease in the neutrophil count, with grade 3 neutropenia in two cases, but there was no temporal relationship between neutropenia and infection. Two cases of AIHA with favourable response to tocilizumab had been previously reported [6, 7], one of them associated with Castleman’s disease, but, to our knowledge, this is the first reported case of a patient with SLE and AIHA refractory to medical treatment with good outcome after adding tocilizumab. Treatment avoided splenectomy as a last therapeutic option. The patient had no infections or other adverse events during treatment. Although larger studies are needed, we believe that tocilizumab is an option to be considered in SLE patients unresponsive to other treatments.

**Rheumatology key message**
- Tocilizumab can be helpful in treating SLE patients with refractory AIHA.

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of-function mutations in the ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene [1–3]. Mutations in this gene are also associated with generalized arterial calcification of infancy (GACI), a devastating neonatal condition resulting in ectopic calcification in the vasculature [4].

We report the case of a 42-year-old Greek female who was referred with a 10-year history of progressive back stiffness and polyarthralgia. She had been diagnosed with HR at the age of 13 months when she developed bowing of her legs. She received vitamin D and occasional phosphate until the age of 20 years, and underwent femoral and tibial osteotomies as a teenager. She had an uneventful pregnancy at the age of 35 years. Her parents were non-consanguineous with no significant family history. Examination revealed short stature and severely restricted spinal movement.

Radiographs demonstrated complete ossification of the anterior spinal ligament, iliosacral ligament calcification and Basstrup’s disease (Fig. 1A–C). Biochemical indices were consistent with HR, and fibroblast growth factor (FGF)-23 levels (post-treatment) were normal at 76 RU/l (<100). Calcitriol 1 μg/day rapidly improved her polyarthralgia; parathyroid hormone (PTH) corrected and other indices remained stable. A short trial of phosphate was discontinued due to intolerance and hyperparathyroidism. At 5-year follow-up spinal stiffness was unchanged, but there was radiological worsening of ligamentous ossification.

Genetic screening failed to demonstrate a PHEX mutation using standard PCR conditions, exon-specific primers and automated sequencing (ABI3730; Life Technologies, Carlsbad, CA, USA; Mutation Surveyor; SoftGenetics, State College, PA, USA). PHEX dosage analysis by multiplex ligation-dependent probe amplification (MRC-Holland kit P223-B1; MRC-Holland, Amsterdam, The Netherlands) did not identify deletions or duplications. Sequence analysis of the coding and flanking intronic regions of the FGF23 and DMP1 genes did not identify any pathogenic mutations. Sequence analysis of the ENNP1 gene (25 exons on chromosome 6) identified two variants: a previously unreported missense variant, p.Thr319Arg (c.956C>T), in exon 9 and a heterozygous nonsense mutation, p.Arg782X (c.2344C>T), in exon 23. The p.Arg782X nonsense mutation results in a premature stop codon at 782 (p.Arg782X) and has been reported previously in a patient with GACI [5]. Parental saliva samples showed that the p.Thr319Arg was paternally inherited and p.Arg782X maternally inherited.

The threonine 319 residue is highly conserved across species and the variant is predicted to be disease causing. In silico phenotypic prediction and 3D modelling was carried out by submitting the ENNP1 phosphodiesterase region (residues 191–591) to the Swiss-Model web server. All in silico phenotypic prediction tools reported the p.Thr319Arg substitution as damaging or not tolerated. Three-dimensional modelling based on template 3nqk chain A (mouse autotoxin) (Fig. 1D) showed that Thr319 lies on the surface, near the binding cleft for the adenosine monophosphate (AMP) substrate, and the side chain forms hydrogen bonds with the protein backbone. In the

References