Immunoabsorption and CD20 antibody treatment in a patient with treatment resistant systemic lupus erythematosus and preterminal renal insufficiency

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Treatment options in severe progressive systemic lupus erythematosus (SLE) are limited and associated with serious potential side effects, such as cytotoxicity, immunosuppression or metabolic imbalances. As SLE is characterised by quantitative and qualitative abnormalities of B cell functions, we reasoned that a combination of extracorporeal therapy (immunoabsorption)¹ with a monoclonal antibody (mAb) to CD20 to deplete the circulating B cell pool^{2–4} might be beneficial in the treatment of refractory severe SLE.

A 49 year old white man who was diagnosed for SLE with primary renal, cardiac, and pulmonary disease in 1989, presented in August 2004. The disease was highly active (European Consensus Lupus Activity Measurement (ECLAM) score 5, pancytopenia, raised anti-dsDNA antibodies, markedly reduced serum complement C3 and C4, and increased erythrocyte sedimentation rate) despite aggressive conventional treatment that had included steroid pulse therapy, methotrexate, anti-tumour necrosis factor (TNF) mAbs, and triple treatment with corticosteroids, azathioprine, and oral cyclophosphamide. Stage VI lupus nephritis (WHO) had been diagnosed by histology earlier in July 2003 and the patient now showed progressive deterioration of renal function (decrease of creatinine clearance from 55 ml/min (December 2003) to 20 ml/min, increase of creatinine from 200 µmol/l to 390 µmol/l, and marked proteinuria of 3800 mg/day), as well as signs of cardiac insufficiency (ejection fraction 50%, massively raised NT-pro-brain natriuretic peptide (NT-BNP) of 10 555 pg/ml). The previous medical history of the patient was unremarkable except for hypertension (diagnosed in 1998) and osteonecrosis of the left femoral head in 1998, for which he had undergone hip replacement surgery.

As anti-TNF mAbs (adalimumab in February 2004, infliximab from November to December 2003) had previously had to be stopped shortly after initiation because of allergic reactions with severe hypotension, steroid pulse therapy could not be used because of the history of femoral head osteonecrosis, and cyclophosphamide had to be discontinued because of severe persistent neutropenia, we decided to treat the patient with a combination of immunoabsorption¹ and anti-CD20 mAb (fig 1).^{2–4} Immunoabsorption was carried out on four consecutive days with a synthetic peptide (GAM146) immobilised on Sepharose CL4B, which typically reduces serum immunoglobulin (IgG 70%, IgM 50%). Anti-CD20 mAbs (375 mg/m²) were given once at the end of each 4 day cycle, and the treatment was repeated every 4 weeks.

After six cycles of combination therapy, clinical activity was well controlled, as documented by an ECLAM score of 2, normal levels for circulating thrombocytes and leucocytes, and normal C3 and C4 levels. Cardiac function was improved (ejection fraction 55%, NT-BNP 1818 pg/ml), as was the renal function (25% increase of the creatinine clearance). The patient tolerated the treatment well with no apparent side effects. Importantly, renal and cardiac functions have been stable now for more than 6 months, and disease activity is

controlled by 5 mg/day corticosteroids and 2 g/day mycophenolate mofetil only.

A recent report indicates that anti-CD20 mAb alone in the absence of conventional immunosuppressant drugs might be insufficient to control severe SLE with glomerulonephritis.⁵ Thus, although we cannot discern precisely the individual contribution of the two immunosuppressive regimens employed here to the clinical outcome in our patient, our data suggest that anti-CD20 mAb together with extracorporeal immunoabsorption might be a promising approach to treatment in severe SLE.

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Figure 1 Renal function in a patient with severe treatment refractory SLE before and during combination therapy with immunoabsorption and anti-CD20 mAb. The arrows denote treatment cycles with immunoabsorption and anti-CD20 mAb. Indicated are serum creatinine levels (line) and creatinine clearance (bars).

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Decrease of regulatory T cells in patients with systemic lupus erythematosus

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Current evidence indicates that regulatory T cells (Tregs) actively suppress autoreactive lymphocytes that escape central tolerance.^{1,2} Tregs are characterised by a high intensity CD25 constitutive expression³ on the surface of CD4 T lymphocytes, which distinguishes them from the non-regulatory CD4+CD25+ T lymphocytes.⁴ Because of their important role in the maintenance of tolerance, it has been suggested that Tregs are decreased in patients with systemic lupus erythematosus (SLE).⁵ We have quantified Tregs in patients with SLE and controls and evaluated their association with disease activity and treatment.

Thirty three patients with SLE (26 women, 7 men) and 14 healthy volunteers matched for age and sex (11 women, 3 men) with a mean age of 39.9 and 36 years, respectively, were studied. All patients fulfilled at least four of the American College of Rheumatology 1982 revised criteria for the classification of SLE.⁶ Disease activity at the time of blood sampling was assessed using the SLE Disease Activity Index (SLEDAI).⁷ Seven patients had inactive disease (SLEDAI = 0), and the remaining 26 patients had a SLEDAI ranging from 1 to 12 (median 4).

Approval from our institutional ethics committees and informed consent from all patients and controls were obtained.

Tregs were identified in peripheral blood mononuclear cells from patients with SLE and controls by direct triple staining immunofluorescence with fluorescein isothiocyanate-antiCD4, phycoerythrin-antiCD25 (both from BD Biosciences), and tricolour-antiCD8 (Caltag) monoclonal antibodies. Data acquisition and analysis were performed with the CellQuest and Paint-A-Gate software, respectively, on a FACSsort flow cytometer (BD Biosciences). Lymphocytes (10^4), gated according to forward and size scatter criteria, were analysed, and the percentage of CD4+ T cells with the brightest CD25 expression was calculated.

The Mann-Whitney U test and the Kruskal-Wallis test were used to compare two or more groups, respectively. Quantitative correlation between variables was assessed by Spearman's rank correlation.

No differences were found in the mean (SD) CD25 expression on CD4+ T lymphocytes between patients with SLE and controls (37.8 (11.9) v 31.3 (9.12), $p = 0.077$) and no correlation was detected between the SLEDAI score and the percentage of these cells in patients with SLE (data not shown). However, patients with SLE had a smaller percentage of CD4CD25bright T cells than controls, in comparison with both the total lymphocytes and the CD4+ T cells (1.29 (0.62) v 3.12 (1.41), $p < 0.001$; 4.11 (1.93) v. 8.24 (2.93), $p < 0.001$, respectively) (fig 1A). In addition, the SLEDAI score and the percentage of these cells were inversely correlated ($r_s = -0.611$, $p < 0.001$) (fig 1B).

To assess whether the treatment the patients were receiving influenced the percentage of CD4CD25bright cells,

patients were classified in five groups: (1) no treatment; (2) antimalarial drugs; (3) antimalarial drugs and low dose steroids (≤ 15 mg/day); (4) immunosuppressive drugs (in combination with antimalarial drugs and/or low dose steroids); (5) antimalarial drugs and high dose steroids (prednisone 30 mg/day). Treatment groups with fewer than three patients were excluded from the statistical analysis. All groups, including patients not receiving treatment, had a lower percentage of CD4CD25bright T cells than controls ($p < 0.001$) (fig 1C); however, no differences were found among the groups of patients ($p = 0.11$).

To date there is no consensus about the role of Tregs in the pathogenesis of SLE. Some authors have found that Tregs are decreased in patients with SLE but that they did not correlate with disease activity.⁸ However, Crispin *et al* showed that only patients with active SLE had a decreased percentage of CD4+CD25bright T cells.⁹

Our results suggest that patients with SLE have a lower percentage of CD4CD25bright T cells, which decreases as the disease activity increases and which is independent of the treatments they are receiving at the time of the study. These observations support the hypothesis that Tregs might be involved in the pathogenesis of SLE.

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