Immunoadsorption onto protein A induces remission in severe systemic lupus erythematosus

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Abstract

Background. Reduction of pathological autoantibodies and circulating immune complexes can be useful in the treatment of autoimmune disease. Plasmapheresis has been shown to reduce autoantibody levels in systemic lupus erythematosus (SLE), but its effect on patients' outcome was not better compared with conventional immunosuppression in the past.

Aim of the study. Immunoadsorption as a selective extracorporeal immunoglobulin elimination technique was evaluated as rescue therapy in patients suffering from SLE.

Methods. Eight patients with severe, therapy-resistant SLE underwent immunoadsorption onto protein A sepharose without concomitant immunosuppressants.

Results. Remission of the disease was achieved in seven patients. Therapy had to be stopped in one patient because of side-effects. The best results were obtained when immunoadsorption was carried out daily, without supplementary intravenous immunoglobulin therapy. Oral cyclophosphamide for 3–6 months during follow-up was used to suppress relapse. Autoantibodies and circulating immune complexes were effectively eliminated regardless of their IgG subclass.

Conclusion. Immunoadsorption onto protein A might be used as an extracorporeal treatment option in SLE when other therapies are ineffective.

Keywords: autoantibody; immune complexes; immunoadsorption; immunoglobulin subclasses; lupus nephritis; protein A

Introduction

Major organ system involvement in systemic lupus erythematosus (SLE) may occur in the heart, lungs, kidneys, and central nervous system and is, together with infections, responsible for most of the mortality and morbidity [1,2]. Recently, intermittent intravenous (i.v.) cyclophosphamide in the treatment of severe SLE with renal involvement or systemic vasculitis was shown to reduce proteinuria in only six of 14 patients while four patients progressed toward renal failure [3]. Since circulating autoantibodies and immune complexes may play an important role in the pathogenesis of SLE, an extracorporeal method for the removal of these pathological antibodies and complexes may be effective in the treatment of active lupus disease. In the past, plasmapheresis has been suggested as an appropriate tool for the elimination of these antibodies or circulating immune complexes but has not proved effective [4–7]. Immunoadsorption onto staphylococcal protein A (SPA) sepharose may eliminate antibodies and immune complexes more efficiently than simple plasma exchange [8]. This paper demonstrates the effect of immunoadsorption onto SPA in eight cases of severe, therapy-resistant SLE. We further investigated the impact of immunoadsorption on the kinetics of immunoglobulins and their subclasses, autoantibodies, circulating immune complexes, and on clinical parameters.

Subjects and methods

Patients

Eight patients (five female, three male) with SLE diagnosed according to the ARA criteria, who were either resistant to conventional treatment (prednisolone, cyclophosphamide) or had contraindications for immunosuppression with cyclophosphamide, were considered for a treatment trial with extracorporeal immunoadsorption onto staphylococcal protein A (Table 1). While all patients had proteinuria, two patients (nos 1 and 8) suffered also from renal failure. Low platelet counts were found in patients nos 6 and 8. Lupus activity was assessed using the SLE activity measure (SLAM) [9]. Written informed consent was obtained from all patients and the study was performed in accordance with the regulation of the local ethics board.

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<table>
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<th>LN WHO class</th>
<th>ANA</th>
<th>Anti ds-DNA Ab</th>
<th>Creatinine</th>
<th>Other autoantibodies</th>
<th>Extrarenal manifestation</th>
<th>Pre-treatment</th>
<th>Total plasma vol. processed (litres)</th>
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<td>Vas, cor, CNS, liv</td>
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</table>

SLAM, systemic lupus erythematos activity measure [9]; LN, lupus nephritis; n/a, not available; ANA, antinuclear antibodies; homo, homogeneous; speck, speckled; Ab, antibodies; max, maximum during active disease; cor, cardiac involvement; pulmo, pulmonary complication; arth, arthritis; CNS, cerebral manifestation; vas, vasculitis and thrombosis; liv, liver involvement; Pred, prednisolone; Aza, azathioprine; CyP, cyclophosphamide; Px, plasmapheresis with cyclophosphamide; C1q, hydroxychloroquine; Ig, immunoglobulins.
Immunoadsorption in systemic lupus erythematosus

Immunoadsorption was carried out as previously described [10]. In brief, blood was drawn from the patient by using a double-lumen jugular or subclavian catheter and plasma was processed over either of two SPA sepharose columns (Imunosorba, Excorim, Lund, Sweden) using a plasma flow monitor (Citem 10, Excorim, Lund, Sweden). Immunoadsorption was started with one relative body plasma volume (BPV) and adjusted according to the elimination rate of IgG, aiming at complete removal after the end of the procedure. Six patients were treated every other day, but patients nos 6 and 8 were treated every day. No immunosuppression or cytotoxic drugs were administered at the beginning of the immunoadsorption for 3 weeks (induction period). The patients were put on oral cyclophosphamide for 3–6 months when remission had been obtained. Patients nos 4 and 6 received i.v. cyclophosphamide on the recommendation of their physicians.

Laboratory investigations

Venous blood samples were drawn before, during, and after the immunoadsorption for blood counts, biochemistry, coagulation parameters, complement fractions C3 and C4, immunoglobulins IgG, IgM, and IgA. IgG subclasses were detected by a sandwich enzyme-linked immunosorbent assay (ELISA) (The Binding Site, Heidelberg, Germany) and results of random samples were confirmed by radial immunodiffusion (The Binding Site). C1q- and C3-fixing circulating immune complexes were determined by ELISA (IBL, Hamburg, Germany). Antinuclear antibodies were detected by standard immunofluorescence test. *Crithidia luciliae* antigen titres (Dr W. F. Gross, Bad Bramstedt, Germany) were tested before and after completion of immunoadsorption therapy. Anti-ds-DNA antibodies were tested by ELISA (Elias, Freiburg, Germany). Anti-ds-DNA IgG3 antibodies were detected by a double sandwich ELISA using ds-DNA coated immunoplates (Elias) and a polyclonal sheep anti-human IgG3 serum (kindly provided by Dr Bradford, Birmingham, UK) and a peroxidase-conjugated anti-sheep antibody (Dianova, Hamburg, Germany).

Results

All patients except one (no. 5) completed induction immunoadsorption. Mean follow-up after beginning of immunoadsorption was 54 ± 10 months. Between four and 17 single immunoadsorption sessions were performed. Total processed plasma volume ranged between 26.5 and 113.7 litres (mean 69 ± 30.4 litres).

Clinical efficiency

Lupus activity measured by the SLAM index decreased from 23.8 ± 4.2 at the beginning of immunoadsorption to 7.9 ± 4.3, *P* < 0.0001 (Figure 1a). Overall daily prednisolone requirement was reduced from 41 mg/day ± 51 to 18 mg/day ± 15, *P* = 0.26. The mean cumulative monthly prednisolone intake before immunoadsorption was 1219 ± 1502 mg compared with 549 ± 452 mg 1 month after immunoadsorption (Figure 1b). Symptoms of polyserositis and arthritis subsided completely during immunoadsorption but a relapse was noted in one patient (no. 4) who experienced severe pericardial effusion just prior to her second i.v. cyclophosphamide dose. Two patients (nos 1 and 8) had dialysis-dependent renal insufficiency which completely subsided during immunoadsorption or shortly thereafter. Two other patient (nos 5 and 7) had a transient increase of serum creatinine, but values returned to normal during immunoadsorption. Overall mean serum creatinine declined from 2.3 ± 2.1 mg/dl to 0.9 ± 0.2 mg/dl (Table 1). Proteinuria declined in all patients during immunoadsorption and no relapse was observed after the treatment (Figure 1c). Patient #1 presented with scintillating scotoma and blurred vision.
Funduscopy demonstrated optical disk oedema, arteriolar wall damage with retinal haemorrhages, and soft exudates. The lesions dissolved completely during immunoadsorption [10].

**Immunological results**

All patients were Crithidia antigen positive at the beginning and became negative during or shortly after ending immunoadsorption. Anti-ds-DNA antibody levels declined although the initial levels varied over a wide range (Table 1). The decline of anti-ds-DNA antibodies and anti-ds-DNA-IgG3 antibodies is shown in Figure 2a,b. Six patients exhibited anti-phospholipid antibodies (anti-thromboplastin and anticardiolipin) and patients nos 2, 6 and 8 had anti-phospholipid associated clinical complications (Table 1). In all cases antibody titres were lowered below detection limit (data not shown) but reappeared during cyclophosphamide treatment. An increase in platelet counts was associated with the removal of anti-phospholipid antibodies.

Complement levels were reduced in most patients at the beginning of immunoadsorption and increased to normal or almost normal levels during the period of immunoadsorption. Patient no. 3 had a congenital complement C4 deficiency and only C3 returned to normal values. When C1q- and C3-fixing circulating immune complexes were initially detected, they were eliminated during a single immunoadsorption procedure (published previously [11]).

**Adverse effects**

Immunoadsorption itself was well tolerated by all patients during most of the treatments. Therapy was stopped in only one patient because of deteriorating renal function. In this patient the coincidence of severe respiratory and metabolic alkalosis with supplementation of i.v. calcium and phosphate buffer (used for regeneration of the SPA columns) led to an increase of the activity product for calcium hydrogen phosphate (-logAP 5.8, relative supersaturation 0.9) and octacalcium phosphate (-logAP 43.7, relative supersaturation 1.3) in the urine [12] with subsequent spontaneous tubular crystal formation [13].

**Long-term outcome**

All patients were discharged from the hospital and followed-up on half-yearly intervals. At last follow-up, SLAM index further declined to 4.5 ± 3.6 and none of the patients required cyclophosphamide after conversion to azathioprine or prednisolone monotherapy. Three patients (nos 7, 2, 3) were again admitted to hospital for active lupus disease and treated with pulse prednisolone. No patient went into end-stage renal disease.

**Discussion**

Progress in the therapy of SLE has increased survival during the last 20 years [14]. Satisfactory remission in many patients with moderate and severe SLE can be achieved with i.v. cyclophosphamide and/or methyl-prednisolone. Attempts to improve survival and morbidity in these patients by the combination of cyclophosphamide with plasmapheresis were shown to be ineffective in controlled clinical trials [5,7,15]. The elimination of pathogenic autoantibodies by removal of plasma and substitution of albumin or fresh frozen plasma is neither efficient nor does it suppress the humoral immune response [4,8]. Two problems arise from plasmapheresis in SLE: (i) Plasma exchange leads to an average decrease for immunoglobulin concentration to only 60% per session, and (ii) plasmapheresis is usually carried out on alternate days and limited to one BPV, mainly because of the risk of bleeding due
to the elimination of coagulation factors. In this work we could demonstrate that a new extracorporeal treatment, immunoadsorption onto SPA, is able to induce remission in severe condition of SLE even when other treatment options had been tried unsuccessfully.

Immunoadsorption was first applied to the treatment of SLE in 1979 when Terman et al. [16] tried to remove anti-DNA antibodies from a patient by plasma perfusion over a charcoal device. Others have used immunoadsorption onto dextran sulphate [17,18], phenylalanine [19–21], tryptophan [22], or SPA [23,24] in patients with SLE and other autoimmune diseases. Clinical improvement could be demonstrated in some of these cases but no reports were suitable to clearly identify the benefit of immunoadsorption, since conventional immunosuppression was also given to the patient. When we used immunoadsorption in a treatment-resistant case of SLE where even synchronization therapy did not induce a remission, recovery was brought by immunoadsorption onto SPA irrespective of immunosuppressants [25]. Our experience with immunoadsorption monotherapy in eight cases with SLE showed that all except one patient achieved a complete or partial remission with well-preserved organ function. All patients had been pre-treated with various immunosuppressants and cytotoxic drugs. At the time of the manuscript revision, relapse-free remission has continued in four patients.

Following complete elimination of immunoglobulins by immunoadsorption a rapid redistribution is noted, because around 50% of IgG is distributed in the extravascular space. Repeated treatments were necessary to suppress disease activity. We noted that alternate-day immunoadsorption was not enough to achieve low autoantibody levels, and we started to treat the patients on a daily basis. Running such a protocol with treated plasma volumes of more than 30 l within a week would certainly cause problems if classical plasma exchange were used. It was also shown that SPA has a higher affinity for immune complexes than for IgG [26]. This was confirmed by the observation of rapid improvement when SLE was complicated by vasculitic or immune complex-mediated lesions. Thus, immunoadsorption could augment the clearance of immune complexes of intermediate size, which is delayed in SLE [27], although we cannot exclude the possibility that some of the effects of immunoadsorption in SLE are due to removal of anti-C1q antibodies [28].

Immunoadsorption onto SPA in SLE has been criticized by others [29] because SPA is reported to bind IgG3 poorly; IgG3 is an immunoglobulin subclass that seems to be elevated in SLE [30,31] and lupus nephritis [32]. IgG3 along with IgG1 accounts for the majority of antibody activity to native DNA, Sm antigen, and histones [33]. We tested the elimination of IgG3 anti-ds-DNA antibodies and found similar reduction rates compared to total anti-ds-DNA antibodies. The adsorption of IgG3 to SPA is nowadays well characterized by an alternative binding site which is located in the Fab-region utilizing VH3 chains [34].

Another problem of extensive immunoadsorption is the suppression of humoral immune response leading to a higher susceptibility to viral and bacterial infections. Careful evaluation for clinical signs of infection and adequate antiviral or antibacterial treatment is recommended.

The effect of immunoadsorption is mainly dependent on the quantity of treated plasma, the frequency of immunoadsorptions, and the antibody kinetics, which can be influenced by the co-administration of immunoglobulins. Intravenous immunoglobulin application is a treatment modality for SLE [35] and combination of immunoadsorption and subsequent i.v. immunoglobulin therapy was thought to potentiate each other's effect on autoantibody and immune complex formation while avoiding infectious complications. But whenever i.v. immunoglobulins were administered to a patient undergoing immunoadsorption, elimination rate of anti-ds-DNA antibodies and complement levels decreased. In patient no. 8 who received iv immunoglobulins and immunoadsorption on a regular basis, platelet counts first increased when immunoglobulin treatment was discontinued, indicating that high concentrations of administered immunoglobulins compete with low levels of pathogenic autoantibodies for SPA binding sites. We concluded that i.v. immunoglobulins should be avoided when immunoadsorption is selected as treatment for SLE and other autoimmune diseases [36].

In conclusion, immunoadsorption onto SPA is highly effective regarding the elimination of autoantibodies and circulating immune complexes and might induce a remission in patients with severe SLE. It is an acceptable alternative treatment option in patients with SLE when other therapies are ineffective or contraindicated. Whether immunoadsorption onto SPA is more effective than classical cyclophosphamide treatment must be proven by a controlled clinical trial.

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