Selective removal of anti-double-stranded DNA antibodies by immunoadsorption with dextran sulphate in a patient with systemic lupus erythematosus

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SUMMARY A dextran sulphate gel column removed anti-double-stranded DNA antibodies (anti-dsDNA) selectively, efficiently, and safely from the circulation of a patient with systemic lupus erythematosus (SLE). The mechanism of the removal is thought to be due to cross reactivity of anti-dsDNA with dextran sulphate, which has negatively charged units. Selective removal of anti-dsDNA is expected to contribute not only to the treatment but also to elucidation of the pathogenesis of SLE.

The aetiology of systemic lupus erythematosus (SLE) is unknown, but autoantibodies, especially anti-double-stranded DNA antibodies (anti-dsDNA) are thought to play an essential part in the pathogenesis of this disease. Plasmapheresis, which removes these antibodies from the circulation, has been found to be an effective treatment for patients with active SLE.¹ Plasmapheresis, in general, removes plasma non-selectively and often requires the use of plasma substitute, which is expensive and may potentially cause viral hepatitis or acquired immune deficiency syndrome. Selective removal of autoantibodies is therefore preferable.

Anti-dsDNA has been shown to cross react with repeating negatively charged molecules other than DNA.² After confirming that anti-dsDNA also had a high affinity for dextran sulphate, which has repeating negatively charged units, we tried to remove anti-dsDNA from the circulation of a patient with SLE using a dextran sulphate sorbent column, which has been used widely as a specific adsorbent for low density lipoprotein in familial hyperlipidaemia.³

Materials and methods

BINDING OF ANTI-DNA WITH DEXTRAN SULPHATE GEL IN VITRO

Patient plasma (0.15 ml) was mixed with 0.1 ml dextran sulphate cellulose gel suspended in 1.0 ml TRIS-HCl buffer (0.15 M, pH 7.6) and incubated at room temperature for two hours with stirring. The gel was centrifuged, the supernatant obtained, and the remaining gel was packed into a column and washed thoroughly with TRIS-HCl buffer. Bound antibodies were then eluted with 2 M NaCl. The levels of anti-dsDNA and anti-single-stranded DNA (anti-ssDNA) antibody in the supernatant and the eluate were measured.

PLASMA SEPARATOR AND ADSORBENT

As a plasma separator, hollow fibre membrane filters of polysulphone with an average pore size of 0.2 µm and effective surface area of 0.5 m² (SF 05, Kanegafuchi Chemical Industry, Osaka) were used. Liposorber LA40 (Kanegafuchi Chemical Industry), in which 350 ml of dextran sulphate cellulose beads were packed, was used as a specific sorbent gel for anti-dsDNA.

PROCEDURE

Blood was withdrawn at a rate of 80 ml/min by a

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peristaltic pump through a double lumen catheter inserted into the femoral vein of the patient. Plasma was separated at a speed of 25 ml/min and passed through the Liposorber with downward flow. The filtrate was infused into the patient with the blood cell-rich portion. Total extracorporeal circulation volume was about 400 ml. For anticoagulant, heparin was injected at an initial dose of 2000 units and then continuously infused at a rate of 800 units/h. Two litres of plasma were treated for each procedure. This procedure was performed three times a week.

Case report

A 30 year old man was admitted to our hospital on 29 May 1987 for further evaluation of SLE. In October 1986 he had experienced a fever of 40°C with polyarthralgia and malar rash. After a diagnosis of SLE he was given 60 mg prednisolone and subsequently improved. In April 1987 he suddenly had two episodes of syncope attack. On 29 May he visited our outpatient clinic and was admitted for further evaluation. He was 175 cm in height and 65 kg in weight. Physical examination on admission showed no abnormality. On laboratory examination the white blood cell count was $8.8 \times 10^9/l$, red blood cell count $4.8 \times 10^{12}/l$, and platelet count $303 \times 10^9/l$. Biochemical data showed no abnormality. LE test and LE cell phenomenon were positive and a serological test for syphilis was negative. IgA was 2.56 g/l, IgG 14.63 g/l, IgM 0.42 g/l, C3 1.32 g/l, and C4 0.13 g/l. Fluorescence antinuclear antibody was positive at a titre of 1/2560 in a speckled pattern. IgG anti-dsDNA was 149 U/ml, IgM anti-dsDNA 19 U/ml, IgG anti-ssDNA 426 U/ml, and IgM anti-ssDNA 200 U/ml, whereas the normal ranges of these anti-DNA are all less than 100 U/l. Anti-nRNP was positive at a titre of 1/320 by passive haemagglutination test. Anti-Sm antibody was negative. Urine analysis, electrocardiogram, chest x ray, electroencephalogram, spinal fluid analysis, and brain computed tomogram were all normal. He fulfilled the 1982 American Rheumatism Association revised criteria for SLE. The 30 mg of prednisolone that he was taking on admission was decreased to 25 mg at day 14. After a few days he

MEASUREMENT

Before and after the immunoadsorption the total blood cells were counted and biochemical data were measured with a Technicon SMAC-C (Technicon Instruments Corp, Tarrytown, NY). Before and at times when each 0.5 litre of plasma had been treated, plasma was sampled before entering and after leaving the sorbent column. The amount of cholesterol in very low density lipoprotein, low density lipoprotein, and high density lipoprotein was measured by the heparin-Ca precipitation method. IgG, IgA, C3, and C4 were measured by laser nephelometry. Titres of anti-dsDNA and anti-ssDNA were measured by enzyme linked immunosorbent assay (ELISA) using dsDNA and ssDNA, obtained according to the methods described by Aotsuka *et al.*,⁴ as antigens. Immune complexes were measured by a modified Clq solid phase binding test.⁵ Antinuclear ribonucleoprotein antibodies (anti-nRNP) were measured by an ELISA using affinity column purified nRNP as antigen.⁶

Fig. 1 Clinical course and titres of IgG anti-dsDNA antibodies, IgG, and C3.

Table 1 Values of recovery rate (R) of plasma components. Values represent the mean (SD) of three procedures

Plasma component	R*
IgG anti-dsDNA	0.596 (0.099)
IgM anti-dsDNA	0.661 (0.050)
IgG anti-ssDNA	0.672 (0.093)
IgM anti-ssDNA	0.658 (0.053)
Immune complex	0.637 (0.099)
Anti-nRNP	0.919 (0.064)
IgG	0.945 (0.073)
IgM	0.965 (0.052)
IgA	0.951 (0.047)
C3	0.927 (0.040)
C4	0.870 (0.090)
Total cholesterol	0.537†
Triglyceride	0.262
HDL‡-cholesterol	0.800
LDL‡-cholesterol	0.527
VLDL‡-cholesterol	0.274
Albumin	0.971

*R is the recovery rate calculated as the ratio of final to initial concentration.

†Standard deviation is not given where there was only one measurement.

‡HDL=high density lipoprotein; LDL=low density lipoprotein; VLDL=very low density lipoprotein.

began to suffer from polyarthritis. On day 27 he developed a fever of 39°C. Increase of the anti-dsDNA titre and decrease of serum complement were also noted. Sixty milligrams of prednisolone was given and the fever subsided. Polyarthritis, however, did not ameliorate and the titre of

anti-dsDNA remained high despite treatment with 60 mg prednisolone and 100 mg of azathioprine for one month. After confirming that his anti-dsDNA bound to dextran sulphate in vitro, we gave immunoadsorbent treatment using the Liposorber, with the patient's agreement.

Results

A study of the binding of anti-DNA with dextran sulphate gel in vitro showed that the titres of anti-dsDNA and anti-ssDNA in the supernatant of the dextran sulphate gel were 22.3% and 34.2% that of the original plasma respectively, whereas those of the eluate were 71.0% and 63.5% respectively.

Figure 1 shows the clinical course and the level of IgG class anti-dsDNA. His anti-dsDNA began to rise as the prednisolone was decreased from 30 to 25 mg. After treatment with 60 mg prednisolone for one month a 30% decrease in the level of IgG anti-dsDNA was noted. Immunoadsorption was able to remove 77.2% of the IgG anti-dsDNA during three procedures over a one week period. There was no rebound phenomenon. Clinical improvement after immunoadsorption was not clear. Fever subsided immediately after treatment with 60 mg prednisolone. Arthritis, which continued even after one month of treatment with 60 mg prednisolone, began to ameliorate on the morning of immunoadsorption. There were no side effects during or after immunoadsorption.

To estimate the capacity of the sorbent column the recovery rate (R) was calculated ($R=C_f/C_i$,

Fig 2 (a) Titres of IgG anti-dsDNA antibodies and trapping coefficient *f* during three immunoadsorption procedures. The *f* value is defined as $f=1-C_a/C_b$, where C_a and C_b are the concentrations of a plasma component after and before the immunoadsorbent column. ○—○=titres of IgG anti-dsDNA antibodies in plasma obtained before the immunoadsorption column; ●—●=those after the immunoadsorbent column; ■—■=value of IgG anti-dsDNA. (b) Amount of LDL-cholesterol and *f* value of first immunoadsorption. ○—○=amount of LDL-cholesterol in plasma obtained before the immunoadsorbent column; ●—●=amount after the immunoadsorbent column; ■—■=value of LDL-cholesterol.

where C_f and C_i are the final and initial concentrations respectively). The recovery rate of IgG anti-dsDNA was 0.596 (SD 0.099) and recovery rates of IgM anti-dsDNA, IgG anti-ssDNA, IgM anti-ssDNA were almost the same as that of IgG anti-dsDNA (Table 1). One session of immunoadsorption was able to reduce anti-dsDNA and anti-ssDNA by about 40% of the original titre. Immune complexes decreased by as much as IgG anti-dsDNA. On the other hand, the recovery rate of anti-nRNP was 0.919 (0.064), indicating that anti-nRNP was scarcely removed. Recovery rates of serum IgG, IgM, IgA, C3, and C4 were almost the same as that of anti-nRNP. Biochemical data, such as albumin, showed no change except for total cholesterol, triglyceride, low density lipoprotein, and very low density lipoprotein.

To evaluate the efficiency of the removal the value of the trapping coefficient f was calculated ($f=1-C_a/C_b$, where C_a and C_b are the concentrations of a plasma component after and before the immunoadsorbent column respectively). The trapping coefficient is 1.0 when adsorption is complete and gets smaller as the capacity for adsorption becomes lower. Figure 2 shows the titre of IgG anti-dsDNA of plasma before and after the immunoadsorbent column, and its trapping coefficient, at times when each 0.5 litre of plasma had been treated (a), and that of low density lipoprotein-cholesterol and its trapping coefficient (b). As the plasma treatment went on the titre of anti-dsDNA in plasma before the column continued to decrease as did that of low density lipoprotein. The trapping coefficient of anti-dsDNA was, however, reduced in proportion to the treated plasma volume, whereas that of low density lipoprotein was unchanged during the procedure.

Discussion

Seventy seven per cent of anti-dsDNA in the circulation could be removed selectively and safely by immunoadsorption using the Liposorber. Anti-dsDNA has been shown to bind not only to DNA, but also to hyaluronic acid, chondroitin sulphate, and heparan sulphate, which contain repeating negatively charged units.² The mechanism of the removal by the Liposorber is, therefore, thought to be by cross reactivity of anti-dsDNA with dextran sulphate, which also has repeating negatively charged units.

Immune complexes were removed as well as anti-dsDNA, though the mechanism is unknown. Anti-nRNP, on the other hand, was hardly removed. Anti-nRNP is thought to be an autoantibody with high specificity in comparison with

anti-dsDNA or rheumatoid factor, and shows no cross reactivity. Plasma immunoglobulin was also hardly removed. As the recovery rate of IgG was 0.945 and the precipitation patterns of IgG in plasma before and after immunoadsorption in immunoelectrophoresis showed little difference (data not shown), the possibility that dextran sulphate removes the positively charged immunoglobulin non-selectively through electrostatic power seems unlikely.

Biochemical data such as albumin, except for total cholesterol, triglyceride, low density lipoprotein, and very low density lipoprotein, showed no significant changes during immunoadsorption. This selectivity made it unnecessary to use plasma substitute. Total cholesterol, triglyceride, low density lipoprotein, and very low density lipoprotein returned to their original levels within a week with no overshoot (data not shown). Such a transient reduction of lipid would produce no clinical problems.

Treatment of two litres of plasma led to the removal of about 40% of the anti-dsDNA. This efficiency is almost the same as that for removal of autoantibodies by plasma exchange.⁷ The value of the trapping coefficient f showed, however, that the capacity of the Liposorber for anti-dsDNA was much smaller than that for low density lipoprotein. Low density lipoprotein, with a molecular weight about 10 times that of IgG, may sterically inhibit the binding of anti-dsDNA to dextran sulphate, or apoprotein B in low density lipoprotein may have higher affinity for dextran sulphate than anti-DNA.

Columns which employ biological substances, such as DNA as used by Terman *et al.*,⁸ have problems for general use. Difficulties include obtaining sufficient amount of material, preventing it from degenerating, and keeping it biologically active as well as the problem of sterilisation. Moreover, these materials may immunise the patients because of their heterogeneity and leak into the circulating blood to form immune complexes. In a study of the selective removal of low density lipoprotein³ dextran sulphate has been shown to have no antigenicity to humans as we also showed in this study.

The removal of anti-dsDNA, which is believed to play a major part in the pathogenesis of SLE, especially lupus nephritis, is expected to be an effective treatment for SLE, even though soluble mediators rather than anti-dsDNA may cause some of the clinical symptoms of SLE. In our patient clinical improvement was not clear after efficient removal of anti-dsDNA by immunoadsorption. This was partly because disease activity had already been suppressed by treatment with prednisolone, but also

the anti-dsDNA removed by the Liposorber might not have played a major part in the clinical symptoms in this patient, who had not suffered from nephritis, and soluble mediators might have exerted a greater effect.

In conclusion, selective removal of anti-dsDNA would contribute not only to the treatment of patients with SLE, but also to elucidation of the precise role of this antibody in the pathogenesis of SLE. Plasma exchange or double membrane filtration plasmapheresis can also reduce anti-dsDNA. In such cases, however, the mechanisms of improvement are not clear because not only antibodies other than anti-dsDNA but also soluble mediators are non-selectively removed from the circulation. As the selectivity of the removal of substances improves the mechanisms of plasmapheresis may also become clear. Application of this immunoadsorption treatment to patients with SLE with greater clinical activity and a high titre of anti-dsDNA in a well controlled study will clarify the true efficacy of selective removal of anti-dsDNA, and we are now carrying out a multicentre trial to examine this aspect. Therefore, the findings reported herein should be viewed as a preliminary basis for future study.

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