Abstract: Immunoadsorption using dextran sulfate (DS)-cellulose columns is reviewed. An extracorporeal selective adsorption system using such columns has been developed and clinically used to remove anti-DNA from the circulating blood of systemic lupus erythematosus (SLE) patients. These columns can adsorb pathogenic anti-DNA subgroups of high avidity and/or cationic antibodies, anticardiolipin, anti-CLβ2GPI, and anaphylatoxins. An open clinical study on 19 SLE cases (the mean number of apheresis sessions totaled 3.7 times; the mean dose of prednisolone, 38 mg/day) revealed that the mean SLE disease activity index (SLEDAI) score significantly decreased from the pretreatment level of 10.2 to 4.5 after treatment. Several case reports have indicated that this modality might also be useful for treating patients with antiphospholipid syndrome. Compartment model analyses showed the one-compartment model to be the most suitable for the kinetics of anti-DNA during and following the apheresis procedure. The indications for immunoadsorption in the treatment of SLE remain controversial. A steroid-sparing effect might be one of them, but further controlled studies are necessary to verify this hypothesis. Key Words: Anti-DNA antibody—Dextran sulfate—Immunoadsorption.

Anti–double stranded DNA antibodies are highly specific for systemic lupus erythematosus (SLE). These antibodies are thought to play an important role in the pathogenesis of SLE. A column packed with beads coupled with dextran sulfate (Selesorb, Kaneka Corporation, Osaka, Japan) has been developed to selectively remove anti-DNA from the blood of patients with SLE (1). The volume of the column is 150 ml.

Anti-DNA antibodies react to polyanionic molecules via the mechanism of cross-reactivity. Aotsuka and Yokohari screened which ligand efficiently adsorbed anti-DNA (2). Among the candidates, dextran sulfate (DS) showed the highest capacity for adsorbing anti-DNA. Kinoshita et al. first reported that they successfully treated an SLE case with a Liposorber column in 1989 (3). Based on these results, the Kaneka Corporation designed a Selesorb column for adsorbing anti-DNA. Using this column, Hashimoto et al. and our group separately performed open clinical studies and thereby demonstrated its efficacy and safety for the treatment of SLE (4–8). This column is now used for the treatment of SLE in Japan. In this article, immunoadsorption using DS-cellulose columns is reviewed.

BASIC CHARACTERISTICS OF THE COLUMN

The differences in the dextran sulfate-bound cellulose beads for the Liposorber and those for Selesorb were as follows: to augment the selectivity of anti-DNA, the pore size of the cellulose beads for the Selesorb was narrowed in order not to be packed by low-density lipoproteins, and the amount of ligand per bead was also increased (1).

The adsorption selectivities of the Selesorb column to the plasma component were as follows: the column efficiently adsorbed anti-DNA (recovery rate in in vitro experiment: 38%), anticardiolipin (53%), and immune complex (74%), while it only slightly adsorbed the total protein (100%), albumin (102%), immunoglobulin G (104%), and C3 (104%) (1).
CLINICAL USE OF THE SELESORB COLUMN

In each apheresis procedure, the twin Selesorb columns are alternately used for adsorbing anti-DNA (6). Owing to the regeneration system, the adsorbing capacity of each column is fully restored every time after the regeneration procedure. The ordinary protocol using the Selesorb columns was first, 4,000 ml of plasma is treated with the columns during each treatment session and second, a total of 4 treatment sessions were performed at 1 week intervals.

The results of an open clinical study on 19 SLE cases which were performed at 4 collaborative hospitals are shown in Fig. 1. The mean number of apheresis sessions totaled 3.7 times, and the mean dose of prednisolone was 38 mg/day. The mean SLE disease activity index (SLEDAI) score significantly decreased from the pretreatment level of 10.2 to 4.5 after treatment, and this level was maintained at least 1 week after the treatment (8).

Two representative SLE cases treated with the Selesorb are shown. One was a 23-year-old active SLE patient who suffered from mild diffuse proliferative glomerulonephritis (9). Four weeks of treatment with prednisolone had little effect on the clinical parameters of lupus nephritis or on the amount of urinary protein. We therefore introduced immunoadsorption. The apheresis had an immediate effect on the reduction of both the anti-DNA and the urinary protein: after 7 sessions, the titer of anti-DNA was reduced from 100 U/ml to 15 IU/ml; urinary protein was decreased from 1.0 g/day to 0.31 g/day. Another case was reported by Daimon et al. from Kanazawa University (10), in which an active SLE patient with diffuse proliferative glomerulonephritis was described. Fifty milligrams per day of prednisolone alone did not suppress the disease activity, so a total of 4 sessions of immunoadsorption was performed. Both anti-DNA and urinary protein were remarkably reduced by the treatment: the titer of anti-DNA decreased from 69 U/ml to 36 IU/ml; urinary protein decreased from 8 g/day to 1.5 g/day, whereas CH50 recovered to the normal range. Besides, they showed that the titer of anti-CLβ2GPI was reduced 4%–65% after each session. After the apheresis sessions, the dose of prednisolone could be smoothly tapered. They performed a renal biopsy both before and after the treatment. Before the treatment, diffuse proliferative changes with PAS-positive massive deposition were noted, whereas the deposition almost completely disappeared after the treatment.

The ratio of the overall improved clinical parameters after the treatment with the Selesorb in the open study was as follows: facial erythema was ameliorated in 87% of the patients; discoid lupus, 100%; arthritis, 80%; proteinuria, 100%, leukopenia, 83%; lymphopenia, 40%; however, no improvement in photosensitivity was observed (8).

After the open clinical study, we performed several additional experiments using the Selesorb. Regarding the adsorbing capacity, we and another group clarified that Selesorb could adsorb a pathogenic population in anti-DNA antibodies, including a high-avidity group (11) and a cationic group (12). In addition, the activated complements and some coagulation factors are also adsorbed by the column (13, 14).

A COMPARTMENT MODEL ANALYSIS

During the open study, we occasionally noted that the levels of anti-DNA as measured by RIA, just after the apheresis procedure was paradoxically larger than those measured just before the proce-
dure. At that time, we speculated that the antibodies in the extravascular pool may have been drawn into the circulating blood both during and after the apheresis procedure, and therefore a two-compartment model is considered to be necessary to accurately interpret the kinetics of anti-DNA (5,6).

To elucidate whether or not the immunoadsorption procedure really provokes a backflow of anti-DNA in the extravascular pool into the circulating plasma, we employed a compartment model analysis.

A 1:100 in vitro scale, single-compartment immunoadsorption system model was devised to analyze the antibody kinetics during treatment (15). In 8 of 10 patients, the log of RIA-measured anti-DNA titers decreased in a linear manner in accordance with the increased treated plasma volume, in both the clinical procedures and the experimental model. These results indicate that a one-compartment model is thus suitable for the antibody kinetics.

However, in one patient, the level of anti-DNA, as determined by RIA, paradoxically increased during the apheresis procedure (15). Accordingly, the anti-DNA titer just after the procedure became larger than that before the treatment. Similar kinetics of anti-DNA were also observed in the in vitro experimental model. In contrast, the level of anti-DNA as measured by enzyme-linked immunosorbent assay (ELISA) decreased in accordance with the increase of the treated plasma in both the clinical and the in vitro model. The samples were diluted at 1:400 and assayed in the antigen double-stranded DNA (dsDNA)–excessive condition in ELISA. In contrast, the plasma samples were diluted at 1:9 and assayed in the condition of comparable antigen:antibody ratio in RIA. We speculate that this paradoxical increase might have been provoked by the presence of specific inhibitors for RIA; they might also have been more rapidly adsorbed by the column than anti-DNA. The inhibitors may not be able to fully coat the excessive dsDNA precoated on wells of the ELISA plate.

Following the apheresis procedure, we also analyzed the kinetics of anti-DNA titers (16). The titers of anti-DNA were measured at specified intervals after apheresis for up to 72 h in each model using nonlinear least-square methods, and Akaike’s Information Criterion was used to determine which model most approximately reflected the kinetics. As a result, the one-compartment model was thus found to accurately reflect the antibody kinetics following the procedure.

### CLINICAL SIGNIFICANCE OF IMMUNOADSORPTION FOR THE TREATMENT OF SLE

The recent “tide” in the treatment of SLE is not favorable for immunoadsorption. Austin and Boumpas established the clinical significance of cyclophosphamide pulse therapy in the treatment of lupus nephritis (17,18). On the other hand, Lewis et al. denied the clinical significance of plasmapheresis in the treatment of severe lupus nephritis (19). Wallace et al. showed that the addition of pulse/synchronization apheresis to cyclophosphamide therapy does not improve the course of patients with proliferative lupus nephritis (20).

What is the significance of immunoadsorption for the treatment of SLE? To reconsider this important theme, I enumerate 3 basic pathological findings in SLE: they are inflammation, cell proliferation, and thrombosis.

According to the published data and our clinical experience, the indications for medication and apheresis for the treatment of SLE are summarized in Table 1. For inflammation, both steroids and apheresis are effective. For cell proliferation, which is typically noted in diffuse proliferative glomerulonephritis, cytotoxic drugs are effective, but apheresis is not effective. For thrombosis (e.g. catastrophic antiphospholipid syndrome and habitual abortion, based on antiphospholipid syndrome), apheresis might also be effective (4,21–23).

The main advantage of cyclophosphamide pulse therapy surely is its ability to reduce the risk of end-stage renal failure while also effectively treating lupus nephritis. However, there are some disadvantages according to the original report (17). It does not reduce the mortality rate in comparison to the prednisone alone-treated group. This therapy also results in a high frequency of premature ovarian failure.

Boumpas described how to treat lupus nephritis at the American College of Rheumatology annual meeting in 1998 (24); however, he did not touch

### TABLE 1. Indications for both medication and apheresis therapy based on the pathological findings of SLE

<table>
<thead>
<tr>
<th>Pathological findings</th>
<th>Effective drugs</th>
<th>Apheresis</th>
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<tbody>
<tr>
<td>Inflammation</td>
<td>Steroids</td>
<td>Effective</td>
</tr>
<tr>
<td>Cell proliferation</td>
<td>Immunosuppressant steroids (?)</td>
<td>Not effective</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>Anticoagulant, antiplatelet steroids (?)</td>
<td>Effective (?)</td>
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upon apheresis therapy at all. Instead, he lectured on how to use corticosteroids: corticosteroids are the first line of treatment for membranous nephropathy with a nephrotic range of proteinuria (1–2 mg/kg q.o.d. for 8 weeks, tapered to 0.25 mg/kg q.o.d. within 3–4 months); corticosteroids may be used in cases of mild to moderately severe proliferative glomerulonephritis (prednisone 1 mg/day for 6–8 weeks followed by tapering to 0.25 mg/kg q.o.d. within 3–4 months); if there is no complete response within 8 weeks, it is necessary to start monthly cyclophosphamide therapy. As described above, massive corticosteroid therapy is still required for the treatment of membranous nephropathy and mild to moderately severe diffuse proliferative glomerulonephritis.

Corticosteroids have many adverse effects, such as osteoporosis, opportunistic infection, peptic ulcer, diabetes mellitus, thrombosis, psychosis, Cushingoid features, hypertension, cataract, glaucoma, myopathy, pancreatitis, metabolic abnormalities, and avascular necrosis of the bone (25). Physicians have to keep in mind that some of these side effects can become overt several months to several years after their massive administration. The steroid dose should be reduced to the minimum needed to treat SLE.

Regarding the significance of apheresis for the treatment of SLE, I would like to enumerate the following points. A steroid-sparing effect can occur if the steroid dose is given in the active phase of the disease. Boumpas proposed that massive corticosteroid therapy should be used for some WHO type IV and V lupus nephropathies which can be reduced by adding apheresis therapy; then, the quality of life afterwards should be remarkably improved. The suppression of inflammation can be achieved as well as a decreased hospital stay. Treatment of recurrent abortion due to antiphospholipid syndrome may possibly be effective. Case reports have indicated that immunoadsorption with Selesorb might also be a treatment modality for recurrent abortion due to antiphospholipid syndrome (4,21–23).

We previously reported preliminary data on patients treated with steroids and Selesorb and those treated with steroids alone. In that paper, the number of patients was only 4 and 4, respectively. The anti-DNA and urinary protein levels were comparable between the 2 groups. However, the total steroid doses were comparatively lower, and the length of hospital stay was comparatively shorter in the patients treated with steroids and Selesorb than in the patients treated with steroids alone (7).

Figure 2 illustrates the steroid-sparing effect that results from the addition of apheresis therapy. To further confirm this effect, a properly designed controlled study is necessary.

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