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Rejection associated with early appearance of donor-reactive antibodies after kidney transplantation treated with plasmapheresis and administration of 15-deoxyspergualin

A report of two cases

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Abstract. In two kidney transplant patients, one of whom had panel-reactive antibodies (PRA) before transplantation, a pretransplant negative donor-recipient crossmatch became positive within the 1st week after transplantation. Simultaneously, good graft function deteriorated to a state of anuria. One patient graft biopsy showed a vascular rejection, whilst the other patient biopsy was unrevealing. Both patients were treated with plasmapheresis and a new immunosuppressive drug, 15-deoxyspergualin (DSG). Plasmapheresis was performed for 6 and 9 days, respectively, and DSG was given for 5 days in a dosage of 6 mg/kg body weight per day. One of the patients received methylprednisolone i.v. in addition. During treatment the crossmatch became negative and has since remained that way. In both patients the graft function was restored. No adverse effects were seen from the treatment, except for a slight leukocytopenia and thrombocytopenia.

Key words: Kidney transplantation – 15-Deoxyspergualin, in kidney transplantation – Plasmapheresis, in kidney transplantation

The presence of HLA antibodies against donor lymphocytes shortly after renal transplantation may lead to graft rejection [8, 11, 14]. This chain of events is more common in sensitized patients, immunized by blood transfusion, pregnancy, or previous transplantation [18]. In animal experiments, a new drug, 15-deoxyspergualin (DSG), has shown a suppressive effect on antibody formation and on xenograft rejection in a concordant model [22, 23]. In humans, promising results have been reported in treating acute kidney transplant rejection with this drug [1, 2]. The drug has also been used for the prevention of rejection [10, 15]. Although the precise site(s) of action of DSG on the immune response is not known, in addition to inhibiting T- and B-cell proliferation, inhibition of antibody synthesis and effects on the ability of macrophages to present antigen have been proposed [5, 24]. We present here the course of two kidney transplant patients who developed donor-reactive HLA antibodies and signs of graft rejection after transplantation and who were successfully treated with a combination of plasmapheresis and DSG.

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Patients and methods

HLA-typing, crossmatches, and definitions of panel-reactive antibodies (PRA) status

Crossmatches and HLA typing were carried out with immunomagnetic beads (IM), using a technique developed by the Dynal Company (Dynal, Norway). Screening for antibodies was performed following the NIH technique or the IM method using a panel of lymphocytes from 15–22 HLA-typed donors. Results are expressed as the percentage of positive reactions. Platelet absorption of the sera was performed by mixing 0.5 ml of packed thrombocytes obtained from approximately 20 healthy blood donors. After 60 min of room temperature incubation with occasional shaking, followed by centrifugation (15000 *g*), serum was collected.

Case 1

A 36-year-old woman with diabetic nephropathy started continuous ambulatory peritoneal dialysis (CAPD) in January 1988. Despite two pregnancies, a panel test revealed no antibodies. A kidney transplantation was performed in October 1989 using a kidney from the patient's brother. The preoperative crossmatch against unseparated donor lymphocytes was negative. The patient was given triple drug

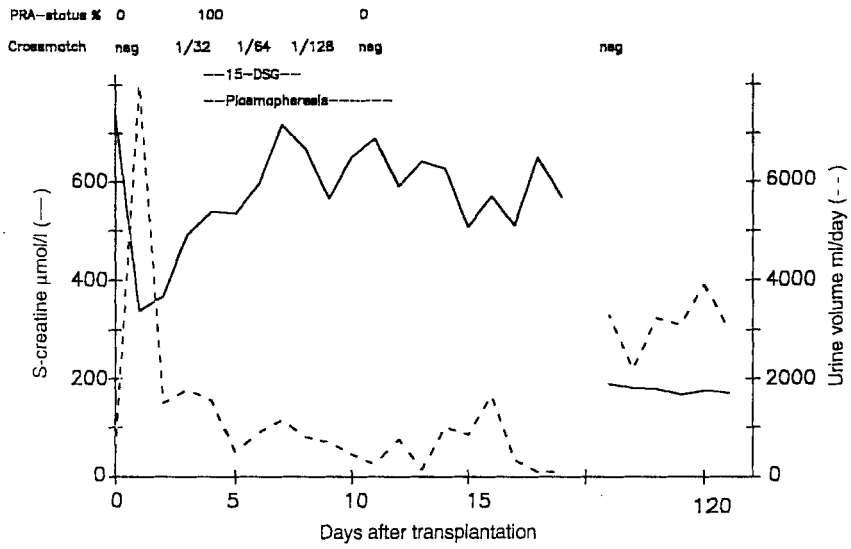

Fig. 1. Postoperative course of patient 1. The patient remained in CAPD for 70 days, but renal function was eventually restored

immunosuppressive therapy consisting of cyclosporin A (CyA), azathioprine, and prednisolone. The graft showed primary function with a rapid decrease in serum creatinine. On the 2nd day after transplantation there was, however, a rapid deterioration in renal function, and a repeated crossmatch was positive at a titer of 1/32 which, 5 days later, reached a maximum of 1/128 (Fig. 1). A core biopsy revealed a severe vascular rejection with fibrin thrombi, edema, and severe tubular necrosis (Fig. 2 a). At this time a panel test showed the patient to have antibodies against 100% of the panel donors.

The patient was treated with daily plasmapheresis for 9 days. The plasmapheresis was performed using a polyethylene membrane filter (Plasmaflo, Asahi Medical, Tokyo, Japan). The daily plasma exchange was 3 l, replaced with Ringer albumin (2.5%) solution. DSG (Behringwerke, Marburg, FRG) was given intravenously (6 mg/kg per day) for 5 days. As a result of the biopsy findings, prophylaxis against vascular thrombosis was also given using low-molecular heparin sodium, (Fragmin (5000 IU daily, given s. c.; Kabi-Pharmacia, Uppsala, Sweden). During this treatment, the crossmatch and PRA status became negative (Fig. 1). A second graft biopsy showed recovery of the severe lesions but some tubular necrosis (Fig. 2 a). For this reason the patient required dialysis for more than 2 months, after which renal function was restored. After treatment with plasmapheresis and DSG, moderate, transient leukocytopenia and thrombocytopenia were seen; no other major adverse effects were noted (lowest recorded levels 2.8 × 10^9/l and 30 × 10^9/l, respectively).

Case 2

A 23-year-old woman with renal failure due to chronic pyelonephritis underwent a second cadaveric renal transplantation in May 1990. She had previously undergone renal transplantation in 1983, but that graft underwent chronic rejection. Since then the patient had acquired PRA against both T and B cells; in the most recent test, the figures were 85% and 95%, respectively. However, the crossmatch against T and B cells from the present donor were negative with current as well as historical sera. The patient received triple drug immunosuppressive treatment as in the previous case. On the 1st day after transplantation there was an adequate urinary output, but the serum creatinine was not reduced. Seven days after transplantation the urinary output ceased and the patient needed dialysis. A fine needle aspiration biopsy did not reveal any signs of rejection. A core biopsy showed minor changes with tubular vacuolization (the patient had serum CyA levels within the therapeutic range and no clinical signs of CyA intoxication). Testing of sera collected on days 6 and 10 revealed a positive crossmatch against donor T and B lym-

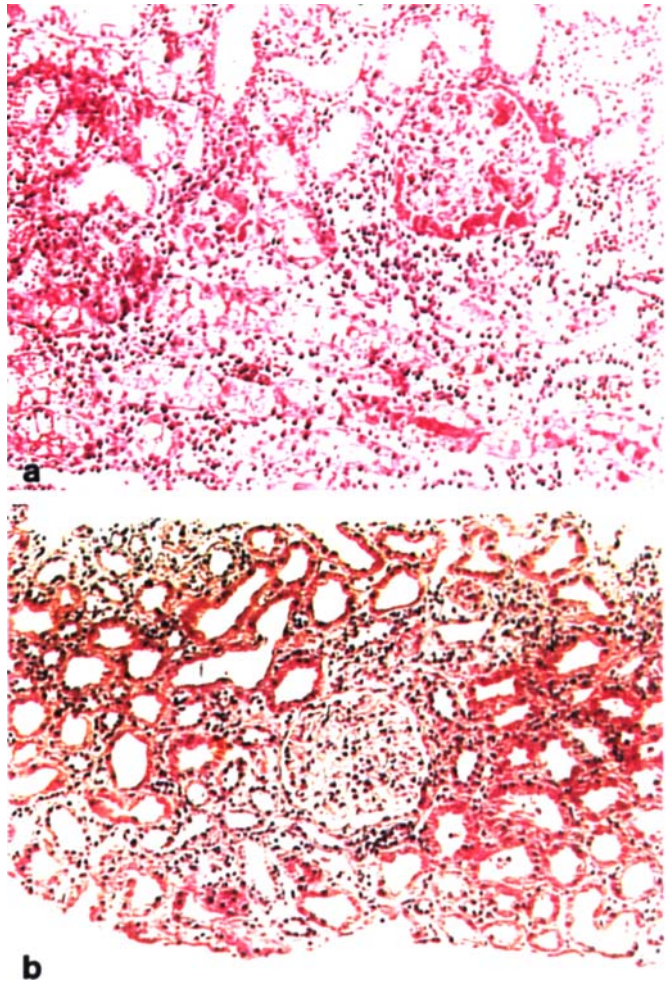

Fig. 2 a, b. Histological appearance before and after treatment with 15-DSG and plasmapheresis in the first patient: **a** before treatment, fibrin thrombi, edema, tubular necrosis, and inflammatory cells are evident in the vascular wall; **b** after treatment, the histology is near normal and these changes are clearly reduced

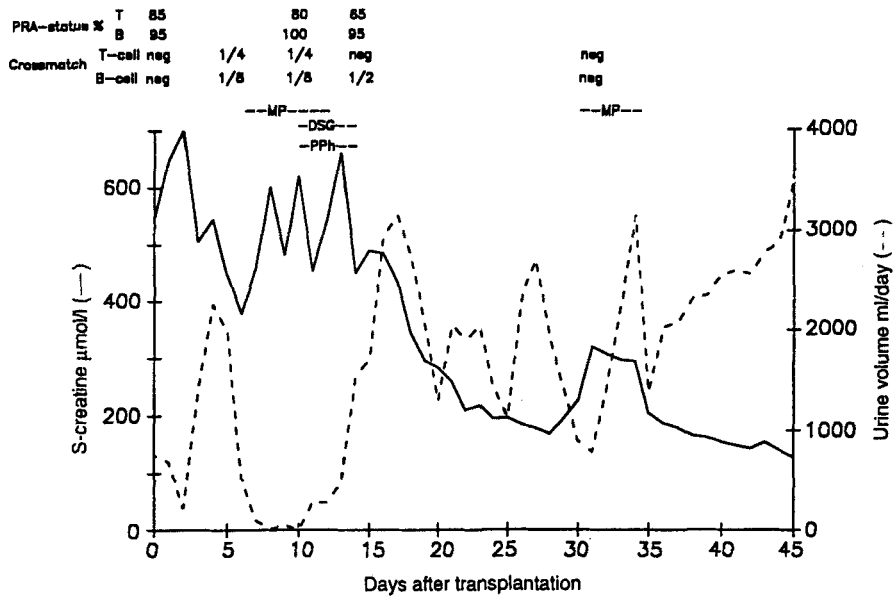

Fig. 3. Postoperative course of patient 2. Note that the crossmatch remained negative during the second rejection episode

phocytes with titers of 1/4 and 1/8, respectively (Fig. 3). All T-cell reactivity and most of the B-cell reactivity disappeared after thrombocyte absorption, indicating that the antibodies were against HLA class I antigens.

Treatment with plasmapheresis was initiated as in the previous case, but with a PF 2000 filter (Gambro, Sweden). Two liters of plasma were exchanged daily. DSG was administered (6 mg/kg body weight) for 5 days. The patient also received i. v. methylprednisolone (Upjohn, Mich., USA) in a total dose of 1250 mg over 4 days. During treatment the T-cell crossmatch again became negative and the B-cell crossmatch after an initial reduction also became negative. The last dialysis on the 4th day after the start of treatment was followed by a full restoration of renal function. One month later a second rejection episode was diagnosed and successfully treated with methylprednisolone (1250 mg/4 days). During this episode, the crossmatch remained negative. As in case 1, the patient developed slight leukocytopenia and thrombocytopenia during DSG treatment, but no other adverse effects were noted (lowest recorded levels 2.3×10^9/l and 58×10^9/l, respectively).

Discussion

The presence of donor-reactive HLA antibodies in recipient serum usually leads to an immediate hyperacute rejection of a transplanted kidney [9, 17]. Antibodies also play a role in acute and chronic rejections [8, 11–14, 21]. During recent years it has been reported that antibodies that do not induce a positive crossmatch in the conventional microcytotoxic assay but that can be detected by flow cytometry may also influence the outcome of renal transplantation. A positive flow cytometric, negative cytotoxic, crossmatch has thus been found to correlate to early nonfunction in the graft and with an increased number of rejection episodes, particularly in retransplanted patients [4, 7, 20, 25]. However, at the time our two patients were treated, this technique was not being used in our laboratories.

In both of our patients, the grafts were initially functional, but within a few days diuresis stopped. Simultaneously, the cytotoxic crossmatch against donor cells became positive in both patients. A histological confirmation of a vascular rejection could, however, only be found in the first patient. Surprisingly, no histological evidence of rejection was found in patient 2. However, the appearance of a positive crossmatch against the donor may mean that deterioration in graft function is associated with immunological status. Both recipients were regarded as immunologically high-risk patients: patient 1 was the mother of two children and, thus, was probably alloimmunized as serum collected after transplantation reacted in a similar manner against nonself antigens from the spouse and panel donors sharing the same implicated antigens. Patient 2 was probably immunized against HLA antigens by a prior kidney transplantation. Both patients were treated with plasmapheresis and DSG, which resulted in the elimination of antibodies, as reflected in a negative crossmatch. Patient continued in CAPD for 70 days, probably due to a severe tubular necrosis caused by the initial rejection. The renal function of patient 2 was restored after a few days.

It is well established that plasmapheresis can be used to reduce the titer of HLA antibodies in patient serum [3, 19]. However, it has proven to be quite difficult to inhibit the resynthesis of antibodies. At least half the patients have a substantial rebound of antibody titers within 1 month, despite intensive treatment with cyclophosphamide [16]. In the two patients studied, no such rebound was seen. This observation implies that DSG was effective in the prevention of antibody resynthesis. This effect was obtained after only 5 days' application, whereas cyclophosphamide treatment is usually applied for several weeks. Cyclophosphamide is also associated with a much higher toxicity than that observed with DSG in our patients and in patients treated by others [1, 2, 10, 15].

Animal and in vitro data indicate that DSG can inhibit antigen-stimulated B-cell proliferation and antibody production; however, DSG does not seem to affect the levels of already existing or preformed antibodies [6, 22, 23]. Therefore, it seems that a combination of plasmapheresis, to reduce levels of existing antibodies, and DSG treatment, to

prevent antibody resynthesis, should be beneficial in patients with early rejection elicited by antidonor antibody formation.

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