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BRIEF REPORT

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Antibody removal and subsequent transplantation of a highly sensitised paediatric renal patient

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Abstract We report a successful renal transplant in a highly sensitised paediatric recipient following removal of HLA-specific antibodies by extracorporeal immunoadsorption. The immediate pretransplant cytotoxic titre against the donor was greater than 1:512; this was reduced to negativity by two immunoadsorption sessions prior to transplant surgery. We also describe the presence of unexpected non-HLA-specific antibody activities in this immunoadsorbed patient.

Key words Kidney transplantation, sensitisation, antibody removal

Introduction

In the South Thames Region of England, 15 % of the adult patients and 33 % of the children awaiting renal transplantation are classified as being highly sensitised to human leucocyte antigens (HLAs) with panel-reactive antibodies (PRAs) greater than 50 %. In 1993 and 1994, highly sensitised recipients received only 5 % of all kidney transplants performed in the region. This means that they are far less likely to receive a transplant than unsensitised patients. These patients have little chance of receiving a compatible kidney graft from a negative-crossmatch donor, as their sera contain alloreactive lymphocytotoxic antibodies directed to a broad range of HLA specificities.

The production of anti-HLA antibodies (sensitisation) can be induced by previous transplantation, pregnancy or blood transfusion; of these, alloantigenic stimulation by a previous graft is the most likely to cause persistently high titres of anti-HLA antibodies. High-titre antibodies to a few HLA specificities may give rise to high PRA titres owing to some of these antibodies crossreacting with other HLA specificities. Therefore, if the crossreactive antibodies that are of lower titre are removed by plasmapheresis or immunoadsorption (IA), then the PRA level will be lowered, and the chances of successful transplantation from a finite pool of potential donors are increased.

To enhance the possibility of these patients receiving transplants, several groups [1, 6, 9], including ourselves, have addressed this problem by removing anti-HLA IgG antibodies from adults before transplantation by extracorporeal IA for which we use protein A sepharose columns. Palmer et al. [6] and Brocard et al. [1] reported the reduction, to crossmatch negativity, of anti-HLA antibodies up to a titre of 1:64 by a course

of IA in a group of adult patients prior to successful transplantation.

This paper reports a renal transplant in a paediatric patient after an IA procedure with a novel protocol. Donor HLA-reactive antibodies were removed from the patient's peripheral circulation at a cytotoxic titre greater than 1:512. This was done in the 36 h prior to transplantation after a donor organ had been identified.

Patient and methods

The patient

A 37.5-kg, 11-year-old boy (blood group O) on haemodialysis had received a mismatched kidney allograft at 2 years of age. The graft was lost 3 years later owing to chronic rejection. In the 6 years between losing his first graft and receiving a second, this patient had been tested against 35 potentially suitably matched donors, all of whom gave rise to positive crossmatches and were therefore contraindicated as donors. The patient's HLA panel-reactive lymphocytotoxic antibodies were persistent and ranged between 75 % and 100 % in the 3 years prior to retransplantation. Continuing problems with arteriovenous access had made this case clinically urgent.

Anti-HLA antibody specificities

The anti-HLA specificities and sensitisation levels of this patient's antibodies were determined by screening monthly serum samples against panels of peripheral blood lymphocytes from 60 normal volunteers of known HLA type selected to cover the majority of known classical HLA class I antigens. A standard NIH complement-dependent lymphocytotoxic test was used.

Immunoadsorption

Extracorporeal IA was carried out with protein A sepharose columns with cyclophosphamide given orally at 2.5 mg/kg per day (the dosage was adjusted to achieve a total white blood cell count of 3–5×10^9/l) as adjunctive immunosuppression to reduce resynthesis of antibodies. The blood was separated into plasma and cellular fractions with a plasmapheresis membrane. The plasma was passed down two protein A sepharose columns (Citem 10 system, Excorim, Lund, Sweden). These were used alternately so that as one became saturated with the patient's immunoglobulin, the plasma could be passed down the second column whilst the first column was being regenerated with an acid wash. After passage down the columns in 10-min cycles at 20 ml/min, the plasma was recombined with the separated cellular components before being returned to the patient.

The mean plasma volume processed in a long IA treatment was 13.6 l, whilst for a short treatment it was 6.3 l. Aliquots of the patient's plasma and serum were taken at hourly intervals throughout the IA procedure and kept at −25 °C for subsequent analysis. The patient underwent several scheduled IA sessions before a potentially suitable kidney became available. Once a suitable kidney had become available, acute IA was carried out immediately before transplantation.

Anti-HLA antibodies

We screened for and excluded non-HLA-specific IgM autoantibodies that would affect the cytotoxic crossmatch (CXM) before we commenced the IA treatment programme. We used the reducing agent dithiothreitol to destroy IgM antibodies whilst leaving IgG antibodies intact in an autolymphocytotoxic test. To measure the efficacy of antibody removal, we screened for anti-HLA antibodies after each planned IA treatment by both cytotoxic and the more sensitive flow cytometric crossmatch (FCXM) on peripheral blood lymphocytes from two normal volunteers. One volunteer was HLA $A2^+$ (mismatched transplant antigen), whilst the other was a non-A2, $A28^+$ ("crossreactive antigen").

For the acute pretransplant IAs, anti-HLA titres were measured against donor spleen cells. Transplantation did not occur until negative crossmatches were achieved by both methods. The PRA level was retrospectively measured with a 60-cell selected panel.

Flow cytometric analysis

We incubated 30 µl aliquots of lymphocytes at 3×10^7/ml with 20 µl of AB serum (negative control) and also with 20 µl aliquots of the patient's serum pre and after each hour of IA. The cells were stained with phycoerythrin (PE)-conjugated anti-CD3 (anti-T cell) monoclonal antibody and fluorescein isothiocyanate (FITC)-conjugated antihuman IgG. Flow cytometric data acquisition and analysis was carried out using a 'FACScan' flow cytometer. The data was gated on $CD3^+$ cells (T cells) and $CD3^-$ cells (B cells) and then analysed for binding of human IgG to the lymphocyte subsets by overlaying the FITC histograms.

Results

Immunoadsorption

The patient underwent seven planned sessions, followed by two acute, pretransplantation sessions of extracorporeal IA. After the first long (8-h) IA session, the cytotoxic titres against both the $A2^+$ cells and the $A28^+$ cells were substantially reduced. Four short (4-h) IA sessions followed. They also produced a steady decline in the pre-IA titres of anti-A2 and anti-A28 (Table 1). A subsequent emergency blood transfusion caused the preimmunoadsorption titres against the A2 and A28 cells to rise: these were reduced following two short sessions of IA.

The first two IA treatments had little effect on the PRAs, but the third treatment reduced the PRA by 25 % (Fig. 1). The two final acute IAs prior to retransplantation reduced the PRAs from 100 % to 56 %. The total immunoglobulin levels were decreased after each IA as shown in Table 1.

The patient underwent two long IA sessions in the 36 h immediately prior to retransplantation with a kidney to which the recipient had a pre-IA cytotoxic antibody titre greater than 1:512. The first treatment reduced the titre to 1:16, and this became negative (CXM and FCXM) after the second treatment (Table 1). A 'rebound' of antibody was seen overnight after the first pretransplant IA. This was probably due to the diffusion of antibodies from the extravascular compartment so that the intra- and extravascular compartments were equilibrated, rather than to de novo antibody production. During the

Table 1 Immunoadsorption data (ND not done)

IA session number	Days prior to transplant	Duration (hours)	Total IgG (g/l)		Cytotoxic titre vs $A2^+$ cells		Cytotoxic titre vs $A28^+$ cells		Cytotoxic titre vs donor cells		PRA (%)	
			pre-IA	post-IA	pre-IA	post-IA	pre-IA	post-IA	pre-IA	post-IA	pre-IA	post-IA
1	−68	8	14.10	1.84	1:128	1:8	1:16	1:2	ND	ND	98	93
2	−57	4	8.03	1.17	1:64	1:4	1:8	0	ND	ND	98	89
3	−55	4	4.01	0.75	1:64	1:4	1:4	0	ND	ND	96	71
4	−51	4	3.79	0.87	1:16	1:4	1:2	0	ND	ND	100	85
5	−47	4	3.68	0.72	1:16	1:2	1:2	0	ND	ND	96	73
After blood transfusion												
6	−37	4	5.19	0.90	1:32	1:2	1:2	0	ND	ND	98	71
7	−8	4	8.46	2.01	1:128	1:32	1:8	1:1	ND	ND	96	100
Immediately before transplant												
8	−1	8	6.15	ND	ND	ND	ND	ND	>1:512	1:16	100	80
9	0	10	ND	0.75	ND	ND	ND	ND	1:32	0	91	56

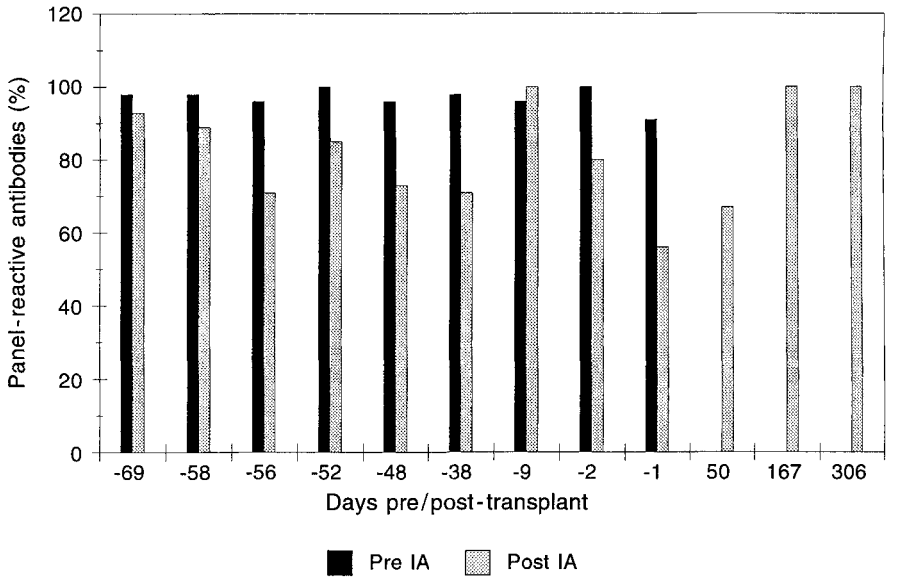

Fig. 1 The effect of the immunoadsorption treatments

long IA sessions, the FCXM profile initially appeared to become more positive, though a subsequent reduction in the binding of antibodies to the donor spleen cells was seen. This patient also demonstrated an 'unmasking' of noncytotoxic IgG autoantibodies after several hours of IA which, whilst not affecting the CXM, does affect the FCXM, giving a false positive result (Fig. 2).

The FCXM mean fluorescence channel shifts, before and after the acute pretransplant IA sessions 8 and 9, against donor T and B cells are shown in Fig. 3. A fluorescence channel shift of 32 channels above the negative control is considered a positive FCXM. The T and B FCXMs were both positive before and after IA session 8. A rebound of antibody occurred prior to IA session 9, with the pre-IA session 9 channel shift being higher than the post-IA session 8 channel shift. However, both the T and B FCXMs were rendered negative at the end of the final IA.

Transplantation

The first kidney allograft (HLA A2,25 B52,18 Cw- Bw4/6 DR1,2) received by this child (HLA A23,33 B42,49 Cw7 Bw4/6 DR4,5) had been mismatched at the HLA A, B and DR loci. He subsequently raised antibodies to the DR2 antigen and all of the mismatched A and B loci antigens, which also crossreacted with other class I HLA antigens. The antibodies to the transplant antigens A2 and B52 were a particular obstacle to retransplantation, as antibodies that also crossreacted with A28 and the whole B5 group had been raised. Owing to the patient's uncommon class I type and his broadly reactive antibodies, any donor kidney that did not repeat the first graft's class I mismatches was considered for transplantation.

Known HLA class I repeat mismatches were avoided with the second donor (HLA A1,3 B57,70 Cw6,7 DR2,7). There was, however, a repeat single class II mis-

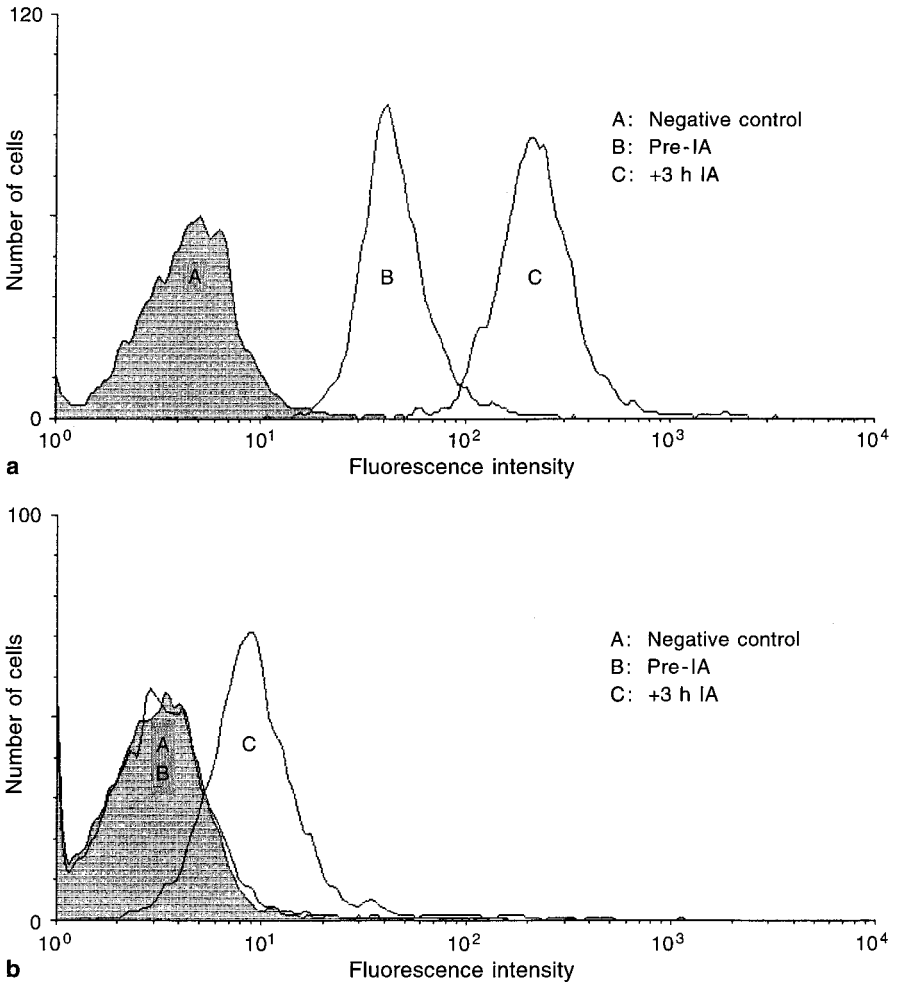

Fig. 2 The flow cytometric analysis of the patient's IgG antibodies against: **a** donor spleen T cells; **b** the patient's own T cells

match (DR2). The cold ischaemia time of the donor kidney was prolonged, (48 h) owing to the time required to achieve a negative crossmatch with IA. In addition to triple therapy (cyclosporin, prednisolone and cyclophosphamide), an initial 10-day course of antithymocyte globulin (6 mg/kg per day, i. v., Fresenius) was given prophylactically with the first dose being given preoperatively. Urine was produced during the first 48 h, but this tailed off and the patient remained anuric for the following 5 weeks. Biopsies, performed at least weekly whilst the patient remained dependent on dialysis, showed severe acute tubular necrosis, but there was no evidence of rejection in these first 5 weeks. However, several mild cellular rejection episodes were subsequently experienced. They were initially treated with i. v. methylprednisolone daily for 3 days. If the rejection episode was resistant to treatment with steroids, a polyclonal anti-T-cell antibody preparation was given (Fresenius ATG, Merieux ATG or ALG). Peritubular capillary thrombi, which were in evidence after the 1st week, were attributed to cyclosporin A nephrotoxicity and were resolved after 5 weeks. Administration of cyclophosphamide was temporarily ceased during the 1st week post-transplant owing to neutropaenia: after 3 months, azathioprine was substituted. Renal function has subsequently improved with a steady decline in plasma creatinine levels (Fig. 4). The patient experienced two urinary tract infections, one severe, at 4.5 and 5 months post-transplant. During the infection at the 4.5-month stage his plasma creatinine rose from 179 mol/l to 292 mol/l within 3 days. The patient responded to treatment with gentamicin and ceftazimide, and his creatinine declined. A biopsy taken at 1 year post-transplant, in response to an increasing plasma creatinine level, did not show any signs of cyclosporin A nephrotoxicity. However, it did show mild cellular rejection, which was successfully treated with steroids. There has been no subsequent sign of rejection. The patient, at the time of writing over 18 months post-transplant, is dialysis-independent, although his plasma creatinine remains relatively high at around 200 mol/l. The current high creatinine level may therefore be due to the patient

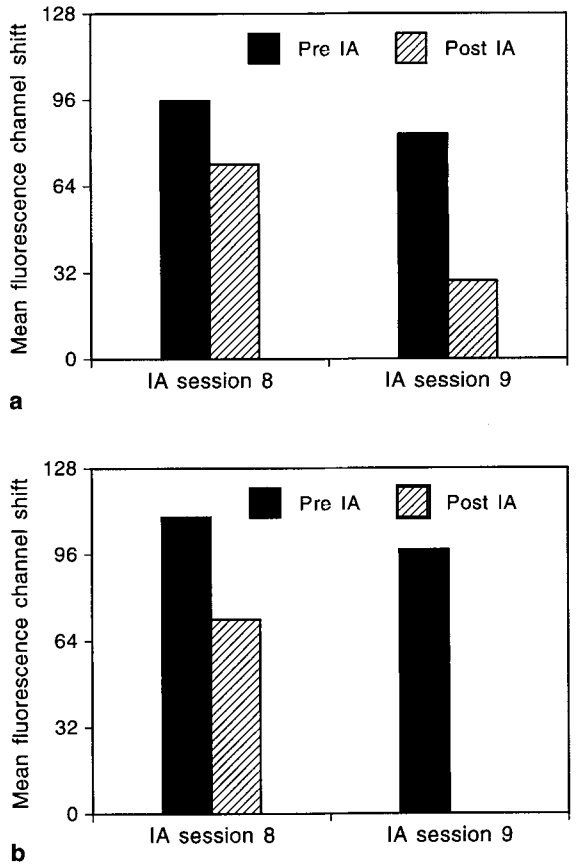

Fig. 3 Plot of flow cytometric crossmatches of the patient's serum before and after acute immunoadsorption (*IA*) sessions 8 and 9 against **a** donor T cells and **b** donor B cells. The values have been corrected for IgG autoantibody binding. A mean fluorescence channel shift (*y*-axis) below 32 is considered negative

failing to completely empty his reconstructed bladder by self-catheterisation and thus suffering intermittent, low-level urinary infections.

Post-transplant monitoring

Post-transplant progress was monitored by biochemical and histological assessment and also by phenotyping of the peripheral white blood cells. The results of the phenotyping were used to assess the efficacy of the immunosuppression and enabled pre-emptive alterations in the level of immunotherapy.

The PRA, having been reduced to 56% immediately prior to transplantation, recovered to its former level of 96% within 6 months (Fig. 1), however, no donor-specific IgG antibodies were produced, as evidenced by the FCXM carried out with stored, frozen donor spleen cells (data not shown). Therefore, whilst anti-HLA antibodies versus third party antigens were produced post-transplant, there was a continued absence of donor specific antibodies in the peripheral circulation.

Discussion

The incidence of highly sensitised patients can be reduced by using HLA-well-matched grafts. However, very few grafts are perfectly matched with the recipient, and some sensitisation may occur. The use of erythropoietin and a subsequent reduction in the number of blood transfusions required by dialysis patients also decreases the risk of sensitisation. Despite these precautions, a cohort of patients remains who have rejected less well-matched kidney allografts and have subsequently become persistently highly sensitised.

This child had produced antibodies capable of binding to all the classical class I antigens as evidenced by his PRA of 100% immediately prior to his acute IA sessions. In addition to antibodies raised to the HLA antigens of the first graft, persistent levels of anti-HLA antibodies were also instituted by therapeutic blood transfusions (data not shown). The persistence and high titre of these antibodies had made it virtually impossible to find a graft for this patient. The anti-HLA antibodies reactive against the second donor were of particularly high titre. These were successfully removed by IA prior to transplantation. We do not know if such a high titre of antidonor antibodies could be removed from the larger plasma volume of an adult. The course of antibody removal by IA cannot entirely be predicted. Therefore, a second potential recipient, crossmatch-negative against the donor, should also be identified in case the immunoadsorbed patient cannot be rendered crossmatch-negative.

During the long IA sessions, a steady reduction in the titre of the cytotoxic anti-HLA antibodies to sensitising and crossreacting antigens was seen by CXM. However, we have made the novel observation that, when assessed by FCXM, there appeared to be an initial and transient increase in the anti-HLA titre. This may result from the depletion by IA of low-titre blocking/anti-idiotypic antibody prior to the significant depletion of the higher titre anti-HLA antibodies. This could cause an apparent increase in anti-HLA antibodies as measured by FCXM, but not by CXM. Anti-idiotypic antibodies to anti-HLA antibodies represent at least one mechanism that down regulates anti-HLA immunity; Reed et al. [8] have reported a good outcome for patients who, at the time of transplantation, had anti-HLA-blocking antibodies. Huestis et al. [5], reporting the exacerbation of a patient's neuropathy following IA, have also speculated on the possibility of the more efficient removal of anti-idiotypic antibodies whilst the majority of the pathogenic antibodies remain within the circulation.

The patient described here was autoantibody-negative when screened prior to commencement of the IA

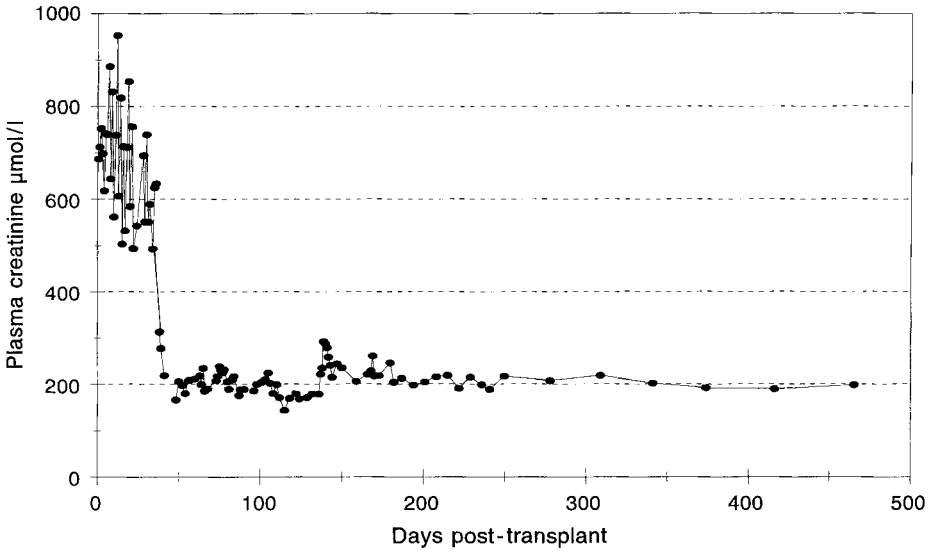

Fig. 4 Plasma creatinine levels

treatment. However, an anti-B-cell, noncytotoxic autoantibody was 'unmasked' during IA. This may have been identified owing to the removal of a blocking antibody, thus allowing the residual autoantibody to bind to the patient's own lymphocytes and be detected.

Autoantibodies reactive with antigenic determinants on the cell surface of autologous and allogeneic lymphocytes are common in humans; cytotoxic antibodies that lyse B cells alone or B and T cells in the presence of rabbit complement have been detected in sera from 20 % of normal individuals [7]. Renal patients with pre-existing autolymphocytotoxic antibodies have also been reported [2, 3]. Ettenger et al. [4] have suggested that the presence of pre-existing cytotoxic autoantibodies may enhance renal allograft survival.

IA has proved to be a useful tool in facilitating the transplantation of highly sensitised patients. High-titre anti-HLA antibodies can be removed prior to transplantation. Care must be taken in interpreting the crossmatch results of immunoadsorbed patients, particularly as a FCXM may become more positive during a conventional short (4-h) session of IA. In some circumstances, short IA sessions may have a deleterious clinical effect.

We feel that this treatment could be made available to other highly sensitised paediatric patients on renal transplant waiting lists.

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