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CASE REPORT

Preoperative treatment of a presensitized kidney transplant recipient with donor-derived transplant acceptance-inducing cells

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Summary

This report describes the case of patient FR, a 31-year-old recipient of a living-related kidney transplant from a donor against whom he was presensitized. Seventeen days prior to transplantation, a central venous infusion of transplant acceptance-inducing cells (TAICs) was administered to the patient. During the 27-month follow up, the patient experienced no acute rejection episodes under an immunosuppressive regime comprising anti-thymocyte globulin (ATG) induction, corticosteroids and tacrolimus. In a similar manner to the kidney transplant recipients treated preoperatively with TAICs in a previous study, patient FR achieved a state of donor-specific hypo-responsiveness. Most remarkably, the deliberate preoperative exposure of a sensitized patient to the sensitizing alloantigen did not heighten his response; on the contrary, after TAIC treatment and transplantation, HLA-specific antibodies were no longer detectable. The case of patient FR provides further evidence of the safety of pre-transplantation treatment with TAICs.

Introduction

Exposure to foreign tissue antigens, as might occur as a result of blood transfusion, may cause patients to develop antibodies against MHC antigens. Potential transplant recipients presensitized to donor HLA in this manner invariably undergo hyper acute rejection, in which the preformed antibodies cause graft failure very shortly after revascularization. In modern renal transplantation practice, the problem of hyper acute rejection has been largely circumvented by careful cross-matching of donor and recipient pairs. However, with the development of more sensitive cross-matching techniques, it is now being realized that a subset of patients have low anti-donor antibody titres, which may not have been detected in conventional cross-matching assays [1]. In these cases, a memory response may be stimulated after transplantation and the patient might experience an early humoral rejection, which is often refractory to treatment with conventional immunosuppression and may result in irretrievable loss of graft function.

The transplant acceptance-inducing cell (TAIC) is a form of deactivated macrophage with immunoregulatory properties [2–7]. Recently published results from the TAIC-II clinical trial suggest that preoperative administration of TAICs to kidney transplant recipients is, at least in some cases, associated with a state of alloantigen-specific hypo-responsiveness or *partial tolerance* [3]. The

concept of partial tolerance has fallen undeservedly into neglect, but usefully describes the beneficial clinical effect of pre-transplantation donor-specific blood transfusion in one haplotype-mismatched donor-recipient combinations [8–11]. By extension, preoperative treatment of transplant recipients with TAICs might facilitate the safe establishment of low-dose maintenance immunosuppression in these patients [3].

This report describes the treatment with TAICs and clinical outcome of patient FR, the recipient of a kidney transplant from a living donor against whom he was presensitized.

Materials and methods

Ethics

TAIC therapy was administered to a single patient in accordance with all the relevant German laws. Ethical approval to undertake an experimental therapeutic procedure in a renal transplant recipient was granted by an independent local ethics committee. The patient gave his full, informed, written consent to the procedure.

Production of TAICs

Human TAICs were prepared as previously described by Hutchinson *et al.* [3]. A total of 4.8×10^9 viable TAICs in 50 ml of 5% human albumin solution were administered to the patient via central venous infusion over 5 min. Prior to infusion, the patient was anti-coagulated with low-molecular-weight heparin.

Immunosuppressive protocol

In addition to treatment with TAICs, patient FR was immunosuppressed with anti-thymocyte globulin, (ATG; ATG-FreseniusS®, Fresenius AG, Bad Homburg, Germany), tacrolimus (Prograf® Capsules, Astellas Pharma AG, Munich, Germany) and prednisolone (Solu-Decortin® H, Decortin H®, Merck Pharma GmbH, Darmstadt, Germany). ATG was administered on day 0, day 1 and day 2 after transplantation. Initially, FR received a combination of corticosteroids and tacrolimus aiming for trough levels of 8–12 ng/ml. Subsequently, corticosteroid and tacrolimus doses were gradually reduced over 30 weeks leaving the patient with a maintenance regime of tacrolimus 5 mg OM and 4 mg ON, plus prednisolone 7.5 mg OD (Fig. 1a).

Immunomonitoring

Patient FR was monitored for indices of transplant rejection and tolerance through the RISET (reprogramming the immune system for the establishment of tolerance) collaborative network, as previously described [3]. For these studies, patient FR was given the code KI/T03/P03.

Mixed lymphocyte culture with multiple cytokine analysis (MLC-MCA)

The MLC-MCA was performed as described elsewhere [3]. Those performing the assays were blinded to the clinical condition of the patient. Proliferative responses have been expressed in terms of the *stimulation index* (the ratio of the stimulated response to the unstimulated (medium-only) response). Quoted values are mean ± SD.

Detection of donor-reactive antibodies

Patients were screened pre- and postoperatively for the presence of HLA-specific antibodies by ELISA and complement-dependent cytotoxicity (CDC) assays. Dithiothreitol (DTT) sensitivity and IgG-specific ELISA were used to discriminate between IgG and IgM antibodies. In the Leiden laboratory, LAT assays were performed for detection of IgG class antibodies to total class I (One Lambda, Montpellier, France) and detection of IgG antibodies against total class II (One Lambda). The specificities of the pre-transplantation antibodies were confirmed in a specific ELISA (One Lambda). The quality of the HLA-specific antibody response is reflected by the strength index, calculated as $10 \times (\text{specific OD1} + \text{specific OD2}) \div \text{cutoff}$, where $\text{cutoff} = (\text{mean of the positive controls} - \text{mean of the negative controls}) \times 0.2 + \text{mean blank signal}$. As a test of the specificity of the loss of HLA antibodies, serum levels of anti-tetanus toxoid antibody were measured by an accredited diagnostic laboratory (Ballies Labor, Kiel, Germany) and values are quoted in IU/ml.

Results

A case study: patient FR

Patient FR, a 31-year-old male patient, was referred to the Transplant Unit at UKSH in Kiel from the University Hospital of Duisburg-Essen as a potential participant in the TAIC-II Study [3]. Patient FR was in preterminal renal failure, because of his underlying diagnosis of tuberous sclerosis. At the time of referral, a living-related kidney transplant from the patient's 58-year-old mother (BR) had been organized. However, the pair was not eligible for inclusion in the TAIC-II study because patient FR was found to have measurable levels of anti-donor HLA antibodies (discussed below). After being declined treatment with TAICs as part of the TAIC-II trial, patient

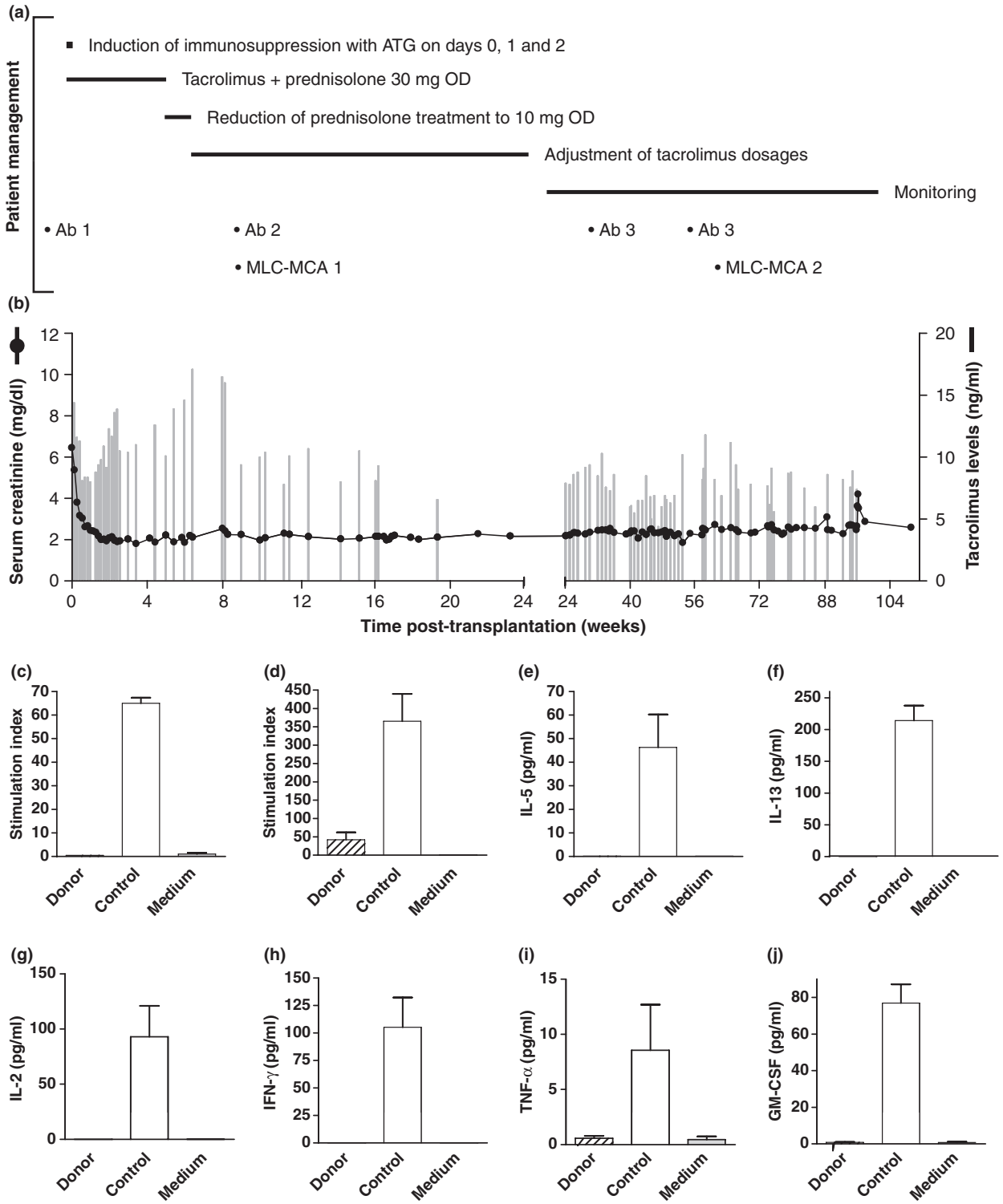

Figure 1 The clinical course of patient FR. (a) Patient FR received immunosuppressive treatment according to the illustrated scheme. HLA-specific antibody screening and MLC-MCA assays were performed at the time-points indicated. (b) Serum creatinine and trough serum tacrolimus levels. (c and d) MLC proliferation assays showed that at week 8 (c) and week 53 (d), patient FR was relatively unreactive to donor stimulation compared to a fully-mismatched 3rd-party stimulator. (e–j) Production of pro-inflammatory cytokines in response to donor-derived stimulators in MLC was minimal, but fully-mismatched 3rd-party stimulation elicited a clear reaction.

FR consented to treatment outside the clinical trial as an individual *healing attempt*.

Patient FR presented with deteriorating renal function after a left-sided nephrectomy for recurrent episodes of pyelonephritis, only 6 months previously. The patient was cognitively normal and there was no evidence of malignancy, or of cardiac and pulmonary associations of tuberous sclerosis. At referral, the patient had a mild normocytic anaemia, but was otherwise haematologically normal. There was no other relevant past medical history.

Donor BR had no past medical history of note. Both the donor and the recipient were blood group A Rh^+ and shared 4/6 HLA matches (Recipient FR: A33, A24, B65, B47, DR13, DR15; Donor BR: A2, A24, B7, B47, DR13, DR15). Donor and recipient were CMV-negative. During the preoperative assessment, patient FR tested positive for anti-donor HLA antibodies in a serological cross-matching assay, and an ELISA-based assay subsequently confirmed that FR had detectable levels of an IgG anti-HLA-B7. Independent verification of these results was obtained from the laboratory in Leiden (strength index of 97), which additionally identified reactivity against B55 and B56. (It should be noted that HLA-B7 carries several different, but highly immunogenic epitopes, which are shared with other antigens, including B55 and B56; therefore, all the detected specificities were potentially donor-reactive.).

According to the TAIC-II study protocol, patient FR received 4.8×10^9 viable donor-derived TAICs (equivalent to 6.9×10^7 cells per kg bodyweight) by central venous infusion 17 days prior to surgery. Administration of the cell infusion was without acute or delayed complications. Explantation of the donor organ and its subsequent engraftment were successful. The warm ischaemia time was 17 min and the cold ischaemia time was 2 h 30 min. Base-line serum creatinine levels of 1.8 to 2.0 mg/dl were attained within the first 2 weeks of transplantation (Fig. 1b).

Initially, patient FR received immunosuppressive therapy according to the TAIC-II protocol: three doses of ATG were administered, followed by a maintenance regime comprising reducing doses of prednisolone and tacrolimus [3]. Prednisolone doses were weaned to 10 mg by the 6th week postoperatively and to 7.5 mg OD by the 31st week. Tacrolimus treatment was reduced over the same period, such that trough serum tacrolimus levels were in the range 6–10 ng/ml between weeks 16 and 34, and further reduced into the range 4–8 ng/ml from week 35 onwards. The patient's graft function remained stable throughout the follow-up period of 110 weeks. Elevated creatinine values in week 99 were because of a right-sided nephrectomy, necessitated by recurrent haemorrhage from renal cysts in the preceding weeks. At the present time, the patient is well, although the rising serum creatinine levels indicate chronic renal dysfunction, most likely because of a chronic rejection process.

The patient's anaemia persisted throughout the study follow up and he became mildly neutropenic. A biopsy revealed a hypoplastic bone marrow, which was not because of CMV infection or non Hodgkin's lymphoma. Importantly, there was no evidence of a graft-versus-host disease-like reaction caused by TAICs or by the lymphocytes contaminating the TAIC preparation. In the absence of an alternative diagnosis, the patient's neutropenia was attributed to his pharmacological immunosuppressive medication.

Loss of HLA-specific antibodies

Screening for HLA-specific antibodies was undertaken at weeks 8, 26, 30 and 53 post-transplantation. At none of these time-points antibodies were detected, either by CDC or ELISA. This loss of HLA-specific antibodies did not reflect a generalized suppression of antibody production as serum immunoglobulin levels were within normal limits. Coincidentally, the patient had been exposed to hepatitis A virus (HAV) in his past and had seroconverted; a virological screen at week 8 showed that the patient remained serologically positive for HAV, suggesting that the absence of HLA-specific antibodies was a specific effect of TAIC treatment or the transplantation. To further demonstrate the specificity of the loss of anti-HLA antibodies, serum levels of anti-tetanus toxoid antibodies were measured at weeks −4, −1, 8, 30 and 53 with respect to the date of transplantation and were found to be relatively unchanged (3.2, 2.7, 2.0, 2.1 and 1.9 IU/ml, respectively).

Special indices of anti-donor unresponsiveness

At the end of the 8th week postoperatively, peripheral blood T cells from patient FR were tested for anti-donor reactivity in MLC (Fig. 1c). Minimal reactivity was observed against HLA-DR-matched stimulator cells from donor BR, but patient FR responded strongly to fully-mismatched 3rd party stimulators. This discrepancy could not be attributed to a low stimulatory capacity of the stimulator cells derived from donor BR because a strong 3rd party reaction against stimulators from BR was observed (data not shown). Neither 3rd party nor FR-derived cells proliferated in the absence of stimulator cells. Comparable results were obtained from the same assay at the beginning of the 53rd week postoperatively (Fig. 1D).

The production of pro-inflammatory cytokines by responder cells from patient FR in mixed lymphocyte cul-

tures against stimulator cells from either donor BR or a fully mismatched 3rd party donor was measured during week 8 (Fig. 1e–j). In response to stimulator cells from the 3rd party donor, responder cells from patient FR secreted substantial amounts of IL-2, IL-5, IL-13, IFN-γ, TNF-α and GM-CSF. However, there was no measurable production of these same cytokines by patient-derived responder cells in response to stimulator cells from donor BR. Similar results were obtained during week 53 (data not shown).

Discussion

Patient FR was transplanted with a kidney against which he had preformed IgG antibodies, but he did not undergo humoral rejection. Unexpectedly, HLA-specific antibodies were no longer detectable 8 weeks after transplantation, an effect which cannot be directly attributed to TAIC treatment, but which runs contrary to clinical expectation. The fact that TAIC administration to a nonimmunosuppressed, presensitized patient did not result in greater sensitization reinforces our view that pre-transplantation TAIC treatment is a safe procedure [3]. Very importantly, this single case study does not prove that TAIC treatment can desensitize a patient, nor can it be claimed that TAIC therapy conferred any measurable benefit to patient FR.

It has previously been observed that TAIC treatment and the technique of preoperative donor-specific blood transfusion might share some mechanistic similarities [2,3]. It has been long-established that preoperative allogeneic blood transfusions given to nonimmunosuppressed patients are associated with a sensitization rate of up to 30%, whereas transfusions given under cover of azathioprine carry a lesser risk of about 7% [11–14]. To date, a total of six patients, including patient FR, have now received pre-transplant TAIC infusions without immunosuppressive cover and none mounted specific antibody responses in consequence. Further studies will be necessary to determine whether the risk of sensitization after TAIC administration is genuinely less than that with pre-transplant, donor-specific blood transfusion.

The case of patient FR does not necessarily imply that TAIC therapy can suppress established humoral immune responses, although we do not discount the possibility that TAICs might somehow influence antibody production. The more important observation, from our perspective, is that the deliberate, preoperative treatment of a presensitized patient with TAICs bearing the sensitizing alloantigen did not heighten the already established antibody response. As TAICs could not be tracked after administration to the patient, and because no biopsies were taken post-transplantation, we cannot know the fate of the preformed donor-specific antibodies: presumably these bound to the infused TAICs or to antigens in the graft itself. We are also presently unable to exclude the possibility that an anti-idiotypic response neutralized the HLA-specific antibodies [15].

Whether or not TAICs are capable of modulating specific B-cell responses (either directly or via a T cell-mediated pathway) is the subject of on-going investigation. Previously, it has been demonstrated that preoperative treatment of presensitized rats with an intravenous TAIC infusion lead to a significant prolongation of graft survival in a heterotopic heart transplant model [5]. In addition, unpublished data show that mouse TAICs can mitigate autoantibody-mediated disease in mice (E. K. Geissler, unpublished data). These experimental data and the case of patient FR suggest potential indications for TAIC therapy, which will be addressed in future clinical studies [6,7].

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Author's contributions

JAH: data analysis, wrote paper. DR: performed MLC analyses, proofread manuscript. MPR: data analysis. BGB-E: trial coordinator. OW: participating clinician. TP: participating clinician. MS: participating clinician. MM: trial coordinator nurse. FG: participating doctoral student. FC: senior scientific participant. UK: principal investigator. EKG: senior scientific participant. FF: senior scientific and clinical participant.

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