Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Transplant acceptance-inducing cells as an immune-conditioning therapy in renal transplantation

James A. Hutchinson,[1] Paloma Riquelme,[1] Beate G. Brem-Exner,[1] Maren Schulze,[1] Martina Matthäi,[1] Lutz Renders,[2] Ulrich Kunzendorf,[2] Edward K. Geissler[3] and Fred Fändrich[1]

1 Laboratory for Transplantation Research, Department of General and Cardiothoracic Surgery, University Hospital of Schleswig-Holstein, Campus Kiel, Kiel, Germany

2 Division of Nephrology, Department of Medicine, University Hospital of Schleswig-Holstein, Campus Kiel, Kiel, Germany

3 Division of Experimental Surgery, Department for Surgery, University of Regensburg, Regensburg, Germany

Keywords
cell-based therapy, macrophage, prope tolerance, TAIC.

Correspondence
Dr James A. Hutchinson, Division of Transplantation Medicine and Biotechnology, University Schleswig-Holstein, Campus Kiel, Arnold-Heller-Straße 7, 24105 Kiel, Germany. Tel.: +49 431 597 2039; fax: +49 431 597 5023; e-mail: james.hutchinson@uksh-kiel.de

Received: 28 January 2008
Revision requested: 2 March 2008
Accepted: 22 March 2008

doi:10.1111/j.1432-2277.2008.00680.x

Summary

The transplant acceptance-inducing cell (TAIC) is a type of immunoregulatory macrophage with the capacity to specifically dampen allogeneic rejection responses to a degree allowing safe minimization of conventional immunosuppressive therapy. In the first part of this report, the production and phenotype of the human TAIC is described. In the second part, an analysis is given of the TAIC-I clinical trial, in which 12 recipients of renal transplants from deceased donors were treated with donor-derived TAICs as an adjunct immune-conditioning therapy. Conventional immunosuppression was gradually withdrawn from 10 of these 12 patients over a period of 8 weeks, starting in the fourth week after transplantation. All but two patients tolerated cessation of steroid therapy, while the remaining eight patients were first weaned from sirolimus and then, in six cases, were also weaned to low-dose tacrolimus monotherapy. It is concluded that TAIC therapy is both safe and clinically practicable; however, the TAIC-I trial was unable to provide evidence that postoperative TAIC administration has a beneficial effect.

Introduction

Studies in animals have defined the essential cellular components of peripheral tolerance and much is now understood of the molecular interactions by which these cell types exert their effects. Purified to homogeneity, tolerogenic populations of dendritic cells and macrophages, and T cells with suppressive function, may suppress allogeneic T-cell responses *in vitro* and this activity, under certain circumstances, may be adoptively transferred into experimental animals [1–8]. Yet, there is still no complete explanation of the role of these diverse cell types in the physiological establishment of peripheral tolerance and no single cell type can be said to be pivotal in this process. More importantly, despite our familiarity with the components of peripheral tolerance, there is no reliable cell-based therapeutic protocol allowing the induction of operational tolerance to allogeneic solid organ transplants.

The possibility of inducing and expanding tolerance-promoting cell types in culture for use as immune-conditioning therapies has received considerable attention [1,9–11]. Opinion is divided about the clinical feasibility of using cultured cells in this way, as the *ex vivo* manipulation of cells is technically demanding and costly, and few centres are equipped for such work. In addition, the potential complications of infusing cultured cells are not insignificant: sensitization against graft antigens, graft-versus-host disease, atypical infections, malignancy and embolism of cellular aggregates are genuine clinical concerns. In favour of cell-based immunosuppressive therapies are their undoubted success in animal transplant models and the advantage that *ex vivo* manipulation allows the composition, quality and dose of the cultured cells to be tightly controlled. Unfortunately, the translation of experimental protocols into the clinical setting has met with only limited success [12,13].

© 2008 The Authors

Work from our laboratories has focussed on the potential of a type of immunoregulatory macrophage, called the transplant acceptance-inducing cell (TAIC), to promote the survival of solid organ allografts. The TAIC was originally identified through studies into the mechanisms by which a rat embryonic stem cell-like cell line promoted the survival of ectopic cardiac allografts in rats [14,15]. Equivalent cell types were subsequently procured from mouse, swine and humans [16–18]. Rat TAICs have been shown to indefinitely prolong heterotopic heart allograft survival in a nonimmunosuppressed Lewis-to-DA transplant model (F. Fändrich, unpublished data) and swine TAICs were shown to prolong lung allograft survival in transplants between outbred pigs after complete cessation of conventional immunosuppression at 28 days post-transplantation (F. Fändrich, unpublished data). On the basis of these preclinical experiments, a series of *individueller Heilversuch* (healing attempts) were undertaken, in which TAICs were used as an adjunct immunosuppressive therapy in patients receiving visceral organ transplants from deceased donors. No evidence of harm to the recipient was observed and a supposed clinical benefit was noted in specific cases [19].

The first part of this report describes the production, morphology and cell surface phenotype of human TAICs. The second part describes the outcomes of the 12 patients enrolled in the TAIC-I trial, a single centre, open-label study of the administration of TAICs for the induction of donor-specific tolerance in renal allograft recipients.

Materials and methods

Preparation of TAICs for clinical application and experimentation

Human TAICs for infusion into patients were generated from donor splenic mononuclear cells under strict GMP conditions, whereas those used experimentally were derived from peripheral blood mononuclear cells under normal laboratory conditions. The method used to produce TAICs from both sources was otherwise identical. Mouse and rat TAICs derived from spleen, bone marrow and blood are phenotypically and functionally identical ([16] and F. Fändrich, unpublished data) and the cell surface phenotype of human spleen and blood-derived TAICs are comparable (data not shown).

The donor spleen was obtained at the same time-point as the transplanted kidney was recovered; this was taken as day 0. To isolate splenic mononuclear cells, the splenic capsule and associated adipose tissue was first removed, before sectioning the spleen into pieces of approximately 1 cm^3. The pieces of spleen were washed thoroughly in Dulbecco's phosphate buffered saline (DPBS) without Ca^{2+}or Mg^{2+} (Cambrex Bioscience, Verviers, Belgium) and were then massaged through sterile filters with a pore size of 0.3 mm to achieve a homogeneous, single-cell suspension. The resulting cell suspension was then layered onto Ficoll (Biochrom AG, Berlin, Germany) in 50-ml centrifugation tubes (Becton Dickinson, Heidelberg, Germany), overlaying 15 ml of Ficoll with 20 ml of cell suspension; these density gradients were centrifuged at 400 *g* for 20 min at room temperature without brake. The mononuclear cell interface was collected by careful pipetting and the recovered cells were washed three times in DPBS (300 *g* for 6 min, then twice at 200 *g* for 6 min).

After isolation, splenic mononuclear cells were suspended in TAIC medium at a density of 5×10^7 cells/ml, and 30 ml of this mixture was added into the requisite number of T175 flasks (Sarstedt, Nürnbrecht, Germany). TAIC medium comprises the following: RPMI-1640 without phenol red containing 25 mM HEPES (Cambrex Bioscience), 10% human AB serum (Cambrex Bioscience), 2 mM L-glutamine (Cambrex Bioscience), 100 U/ml penicillin and 100 μg/ml streptomycin (Cambrex Bioscience) and recombinant human M-CSF (rhM-CSF; R&D Systems, Wiesbaden-Nordenstadt, Germany) at a final concentration of 5 ng/ml, carried on human serum albumin (Aventis, Frankfurt, Germany). The plated cells were then incubated overnight at 37 °C and 5% CO_2.

On the following day (day 1) each culture flask was gently agitated to resuspend the majority of nonadherent cells that had sedimented. The culture supernatants, including the suspended cells, were then transferred to fresh flasks by pipetting, and fresh TAIC medium was applied to the original flasks. All the cell culture flasks were then returned to the incubator.

After overnight incubation, the supernatants from those cultures which had been replated on day 1, including the nonadherent cells, which they contained, were transferred into fresh T175 flasks. The supernatant removed from the flasks was replaced with an equal volume of freshly prepared TAIC medium. All the cultures were again placed in the incubator. For clarity: On day 2, two serial transfers of the supernatant from each initial culture flask had been made, with no cells from the original cultures being discarded.

On day 4, fresh TAIC medium containing a final concentration of 25 ng/ml of interferon-γ (IFN-γ) (Imukin®, Boehringer Ingelheim, Germany) was prepared. Cell culture flasks were gently swung to resuspend the majority of nonadherent cells, but not so vigorously as to detach any loosely adherent monocyte-derived cells. The supernatants were then removed by gentle pipetting and discarded, including all the cells in suspension. The medium in each flask was replaced with TAIC medium supplemented with IFN-γ. All flasks, regardless of the day on which they were plated, were treated in this

© 2008 The Authors

same manner. The cells were returned to the incubator overnight.

Transplant acceptance-inducing cell preparations were harvested on day 5. Each culture flask was gently agitated to resuspend any nonadherent cells. The flask was then positioned vertically and the supernatant aspirated. This supernatant was collected into 50-ml centrifugation tubes. The flask was again laid horizontally and the adherent cells were washed in DPBS without Ca^{2+}or Mg^{2+}, before the adherent cells were gently lifted into suspension in DPBS (without Ca^{2+}or Mg^{2+}) using a standard rubber-cell scraper. Damaging TAICs by scraping is a major determinant of the number of cells ultimately recovered and must be done with great care. We find that leaving the TAICs to rest in DPBS without Ca^{2+}or $Mg^{2+}$for 10 min before scraping results in greater viability and also that small, gentle strokes of the scraper are preferable to long, heavy strokes. TAICs from all flasks were pooled and then resuspended in an isotonic human albumin solution for central venous infusion.

Preparation of resting (M0) and classically activated (M1) macrophages

M1 macrophages were generated from plastic-adherent monocytes cultured for 5 days in phenol red-containing RPMI supplemented with 2 mM L-glutamine, 10% FCS, 100 ng/ml rhM-CSF and antibiotics, before being pulsed for 24 h with lipopolysaccharide (LPS) from Salmonella minnesota at 10 ng/ml (Sigma, Taufkirchen, Germany) and 25 ng/ml IFN-γ. Resting macrophages were produced as per the same protocol as M1 MΦ, except that they were not exposed to IFN-γ or LPS.

FACS staining

Harvested cells were washed twice in ice-cold staining buffer (DPBS with 10% BSA and 0.02% NaN_3) before blocking with 10% FcR Block (Miltenyi, Bergisch Gladbach, Germany) for 30 min on ice at a density of 10^7 cells/ml. Directly conjugated primary antibodies were applied at a final concentration of 1 μg/10^6 cells, unless otherwise directed by the supplier. Antibodies with the following specificities were used: CD14 (catalogue #555399; Becton Dickinson (BD), Heidelberg, Germany), CD13 (BD, #555394), CD33 (BD, #555450), CD11b (BD, #555388), CD11c (BD, #555392), CD66b (Immunotech, #0531; Immunotech, Marseille, France), human leucocyte antigen (HLA)-DR (BD, #555811), CD80 (BD, #557227), CD86 (BD, #555658), CD1a (Immunotech, #1942), CD40 (BD, #555589), CD16 (BD, #555406), CD64 (Iotest, #IM3601), CD163 (BD, #556018), CD205 (BD, #558069), CD206 (BD, #551135), CD30 (BD, #555829), CD38 (BD, #555460), CD69 (#555531), CD71 (BD, #551374), CD18 (BD, #555924), CD54 (BD, #555511), CD56 (BD, #345811), CD62L (BD, #555544) and CD103 (BD, #550260). 7-AAD (BD, #559925) was used for dead cell exclusion. FACS analyses were performed with a BD FACS Calibur machine and data were recorded and analysed with Cell Quest software (BD Biosciences, Heidelberg, Germany). The same instrument settings and gating strategies were used for comparative analyses of M0 MΦ, M1 MΦ and TAICs.

General overview of the TAIC-I study design

A clinical trial protocol for the TAIC-I study was approved by an independent local ethics committee in April, 2003. The TAIC-I trial was conducted in accordance with the Declaration of Helsinki and its revisions, and all relevant German laws, including the regulations of the German Drug Law and guidelines for the clinical testing of drugs. The TAIC-I trial was monitored by an independent agency, Premier Research Germany (Darmstadt, Germany).

The TAIC-I study was a phase I/II clinical trial, taking the form of an open-label, single-centre study. At the time of the TAIC-I trial, there were no safe, established clinical protocols for weaning patients from conventional immunosuppressants. Therefore, the study was without controls.

Patient characteristics

Fourteen patients were considered for enrolment in the TAIC-I study, according to the following inclusion and exclusion criteria. Patients, both male and female, had to meet all of the following inclusion criteria: They had to be aged between 18 and 64 years; receiving their first renal transplant; fulfil the allocation criteria stipulated by Eurotransplant and the Bundesärztekammer; and, give full, informed consent in writing to their participation in the trial. No specific criteria concerning donor organ quality were stipulated. Donor and recipient pairs with all degrees of histocompatibility matching were accepted into the trial, except pairs with complete HLA-matches. Patients were excluded from the TAIC-I trial if they fulfilled any one of the following criteria: if they had an active infection at the time of proposed entry into the study; if they were cytomegalovirus (CMV)-negative and their donor was CMV-positive; if the recipient was presensitized to donor antigens; if they had a history of drug or alcohol misuse; if they were pregnant or nursing mothers; if they had a contraindication to any of the immunosuppressive agents used in the TAIC-I protocol; if they had a past medical history or a current diagnosis of malignancy, vasculitic disease, or any disease requiring systemic steroid therapy.

© 2008 The Authors

Table 1. Clinical characteristics of the TAIC-I patient cohort.

Patient	Age (years)	Sex	Renal disease necessitating transplantation
WW	53	Male	Adult polycystic kidney disease
FK	34	Male	Membranoproliferative glomerulonephritis
DW	56	Male	Type II diabetes and nephrosclerosis
UF	54	Male	End-stage renal failure with no formal diagnosis
MR	35	Male	Hereditary nephritis in Alport's Syndrome
ME	48	Male	IgA nephropathy
MT	38	Male	Unclassified glomerulonephritis
GH	61	Female	Nephrosclerosis
KGW	57	Male	Focal segmental glomerulonephritis
UH	44	Male	Focal segmental glomerulonephritis
GS	45	Female	Membranoproliferative glomerulonephritis
GM	30	Female	Membranoproliferative glomerulonephritis

Of the 14 patients considered for trial participation, 12 were selected for TAIC therapy (Table 1). Nine of the 12 patients were male and all were white-caucasian.

Immunosuppressive protocols and TAIC therapy

All patients entered into the TAIC-I trial received the same immunosuppressive regimen (shown in Fig. 4a). This protocol was based on those of previous studies, which sought to wean patients to tacrolimus monotherapy without adjunct therapies [19–21] and also on the immunosuppressive regimen used in preclinical studies of TAICs in swine (F. Fändrich, unpublished data). As detailed below, patients received triple immunosuppression for the first month post-postoperatively and were then weaned to the same target maintenance level of tacrolimus monotherapy (10 ng/ml) as described by Coupes *et al.* [19]. Only patients who had attained stable graft function with this level of immunosuppression by week 8 were further weaned.

Patients were initially immunosuppressed with tacrolimus (trough level 10–15 ng/ml), sirolimus (trough level 4–8 ng/ml) and glucocorticoids (prednisolone and methylprednisolone). Patients were then weaned from steroid therapy if, on day 28 post-transplantation, the following criteria were met: the patient's serum creatinine was <2.0 mg/dl; there were no biopsy-proven signs of rejection; and the clinical and ultrasound examination of the transplanted organ did not indicate rejection. Steroids were reduced over 14 days in weeks 5 and 6 post-transplantation. If, at the end of the sixth week, the creatinine clearance (CL_{Cr}) was not reduced by ≥25% in relation to the value on the 28th day postoperatively, then sirolimus therapy was gradually reduced over weeks 7 and 8. If, at the end of the eighth week, the CL_{Cr} was not reduced by ≥25% in relation to the value on the 28th day postoperatively, then tacrolimus therapy was reduced over the subsequent 4 weeks (weeks 9–12) to trough plasma levels of 8–10 ng/ml. If, at the end of the 12th week, the CL_{Cr} was not reduced by ≥25% in relation to the value on the 28th day postoperatively, then tacrolimus therapy was further reduced in weeks 13–24, such that trough plasma levels would be in the range 5–8 ng/ml. If, at the end of the 24th week, all clinical measures of graft function were stable, treatment with tacrolimus was to be lowered until serum trough levels were <4 ng/ml and, if it were deemed appropriate by the responsible physician, to complete cessation.

Transplant acceptance-inducing cells were administered on the fifth day postoperatively. The TAICs were resuspended in 50 ml of human serum albumin solution and delivered by central venous infusion over approximately 2 min. All patients received $>1 \times 10^6$ viable TAICs per kg bodyweight, but a standardized cell dose was not stipulated because the optimal cell number was not known and, also, because the TAIC yield varied between donors (Table 2). Patients receiving TAICs were prophylactically treated with heparin.

Statistical methods

In Fig. 2, the mean and standard deviations of mean fluorescence intensities are shown. To compare CL_{Cr} on days 28 or 42 with the number of viable TAICs administered per kg bodyweight to individual patients, an unconstrained linear regression was performed and the coefficient of determination (R^2) has been determined (Fig. 5). A paired *t*-test was used to quote whether patients had significantly lower CL_{Cr} values, or higher serum creatinine levels, at their last clinic follow-up compared to day 28.

Results

Transplant acceptance-inducing cell morphology

The morphology of macrophages in culture depends upon their state of activation, the nature of the surface to which they adhere, and their plating density. Under TAIC

© 2008 The Authors

Table 2. Details of transplant and TAIC treatment.

Patient	Tissue typing			Ischaemia times		No. TAIC administered
	Total HLA mismatches	Class II matches		Warm	Cold	
		HLA-DR	HLA-DQ			
WW	5	0	0	0:25	6:07	Total: 5.8×10^7 per kg BW: 0.55×10^6
FK	4	0	2	0:25	5:50	Total: 1.5×10^8 per kg BW: 1.93×10^6
DW	4	1	0	0:30	5:25	Total: 9.0×10^7 per kg BW: 1.0×10^6
UF	5	0	0	0:19	5:00	Total: 1.94×10^8 per kg BW: 2.69×10^6
MR	5	0	–	0:32	7:02	Total: 5.0×10^8 per kg BW: 7.52×10^6
ME	5	0	1	0:25	3:10	Total: 1.37×10^8 per kg BW: 1.67×10^6
MT	4	1	0	0:25	11:29	Total: 4.25×10^8 per kg BW: 5.26×10^6
GH	5	0	1	0:26	5:38	Total: 4.0×10^8 per kg BW: 5.26×10^6
KGW	5	0	1	0:25	11:05	Total: 3.2×10^8 per kg BW: 4.21×10^6
UH	3	0	–	0:33	8:13	Total: 3.1×10^8 per kg BW: 4.66×10^6
GS	3	1	1	0:23	3:36	Total: 1.5×10^8 per kg BW: 2.08×10^6
GM	5	1	–	0:21	6:20	Total: 3.8×10^8 per kg BW: 7.17×10^6

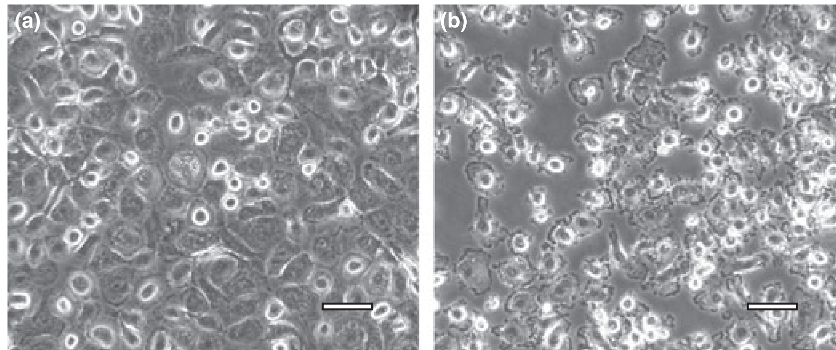


Figure 1 The morphology of TAICs in culture. (a) TAICs adopt an epithelial cell-like morphology with a central body of cytoplasm surrounded by a densely granular skirt of cytoplasm and the margins of adjacent cells in close apposition. (b) Morphologically, TAICs are readily distinguished from classically activated (M1) macrophages (bar = 50 μm).

culture conditions, macrophages adopt a tessellating epithelioid morphology with a prominent central body, usually with a single, clear nucleus, surrounded by a thin skirt of cytoplasm (Fig. 1). The edges of adjacent cells lie in very close apposition and it is often difficult to discern any space between them. The most remarkable morphological feature of TAICs in culture is their size, which is in the order of 50 μm in diameter, depending primarily on the local cell density. TAICs are extremely granular, which may relate to their phagocytosis of cellular debris and co-cultured lymphocytes [16].

The cell surface phenotype of TAICs

The cell surface phenotype of TAICs is consistent with their being a subtype of macrophage: TAICs uniformly express the myeloid markers CD13 and CD33, but not the granulocytic marker CD66b (Fig. 2). All TAICs

© 2008 The Authors

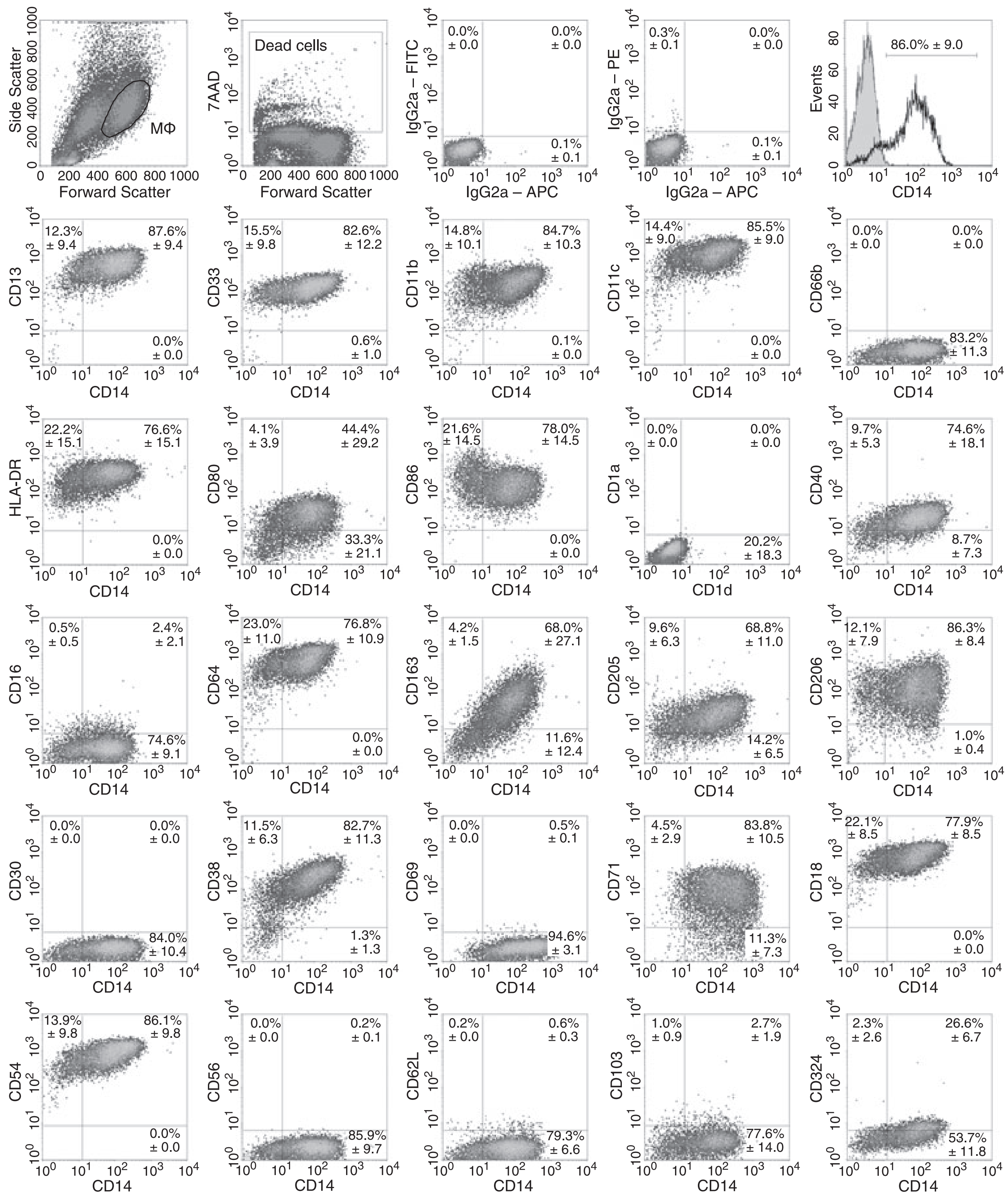


Figure 2 The cell surface phenotype of human TAICs identifies these cells as a subtype of macrophage in a state of partial maturation. Cited values are mean ± SD (n = 3).

express CD205, identifying them as cells of monocytic origin. Variable expression of cell surface CD14 was observed (86.0 ± 9.0%). A very small number of TAICs expressed CD16, but at much lower levels than normally observed on $CD16^{+}$ *resident* monocytes from peripheral blood; in contrast, CD64 expression was high in TAICs.

© 2008 The Authors

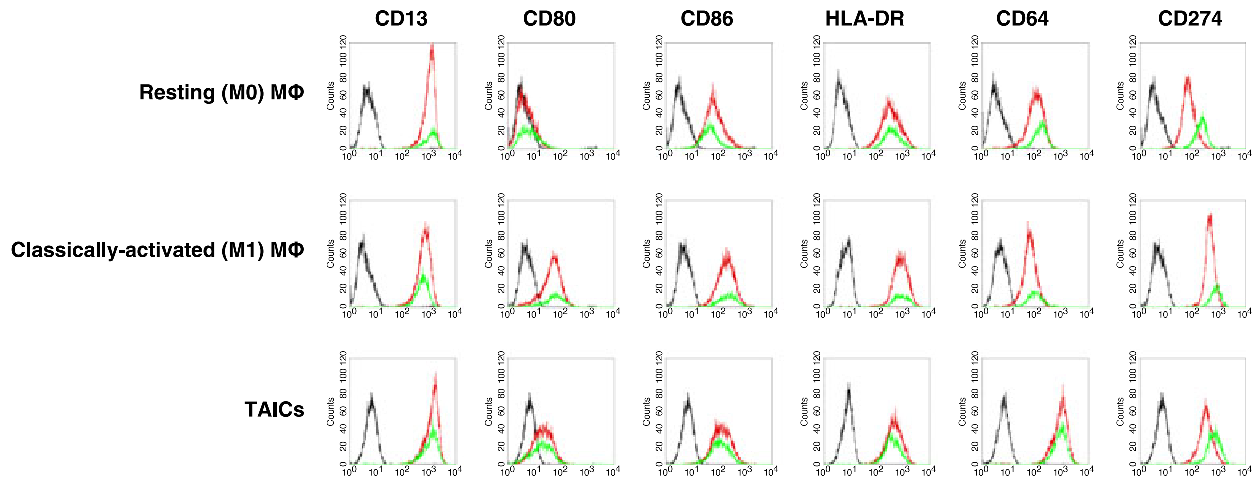


Figure 3 Resting (M0) macrophages, classically activated (M1) macrophages and TAICs were analysed by flow cytometry. TAICs exhibited a partially matured phenotype with higher levels of CD80, CD86 and HLA-DR expression than observed in M0 MΦ, but lower levels than M1 MΦ. Cell-surface expression of CD64 and CD274 (PDL1) distinguishes these three macrophage subsets. Representative plots from two donors are shown (red and green traces) with the relevant isotype controls (black traces).

TAICs uniformly express the macrophage mannose receptor (CD206), whereas CD163 expression is largely restricted to the $CD14^+$ TAIC subset. CD30 cannot be detected on TAICs, but CD38 is expressed at relatively high levels. TAICs do not express the common leucocyte activation marker, CD69, but do express CD71. TAICs express lower levels of HLA-DR, CD80 and CD86 than activated macrophage subsets (Fig. 3). The expression of other macrophage lineage markers allows a phenotypic comparison with the mouse TAICs [16] and also demonstrates the considerable heterogeneity of clinical TAIC preparations, which most likely reflects different stages of TAIC differentiation rather than the existence of distinct cell types. Taken together, these observations identify TAICs as partially matured macrophages, more similar to M1-polarized macrophages than any M2-polarized macrophage subtype [22–26], a phenotype which is not inconsistent with its postulated role in suppression of T-cell responses [27]. This state of partial maturation accords with observations made in the mouse [16].

Recruitment and treatment of patients in the TAIC-I trial

In September 2003 a single-centre, open-label, uncontrolled study of TAICs as an adjunct immunosuppressive therapy in renal transplantation was commenced: this Phase I/II clinical trial was called the TAIC-I study. The primary objective of TAIC-I study was to obtain information on the safety and tolerability of treatment with TAICs. The secondary objective was to monitor renal allograft function and survival after administration of TAICs followed by the controlled withdrawal of conventional immunosuppressive treatment. The TAIC-I clinical protocol is described above and illustrated in Fig. 4a. A total of 12 patients were enrolled into the TAIC-I study and their clinical outcomes are summarized in Fig. 4b.

Figure 4 Overview of the TAIC-I clinical trial. (a) Initially, patients were given triple immunosuppressive therapy comprising tacrolimus (trough levels of 10–15 ng/ml), sirolimus (trough levels of 4–8 ng/ml) and glucocorticoids. If graft function remained stable at the end of week 4, steroid therapy was gradually stopped over the course of weeks 5 and 6. Provided graft function had not deteriorated during steroid weaning, then treatment with sirolimus was withdrawn during weeks 7 and 8. If sirolimus cessation was without adverse consequence, then tacrolimus treatment was minimized over the subsequent 4 weeks, such that trough serum tacrolimus levels were in the range 8–10 ng/ml. Further minimization of tacrolimus monotherapy was undertaken during weeks 13–24, aiming for trough serum tacrolimus levels of 5–8 ng/ml. Patients who presented no clinical signs of rejection and had normal graft histology at the end of week 24 were considered for further reduction of tacrolimus treatment. (b) Twelve patients were enrolled in the TAIC study and received TAIC therapy, 10 of which qualified for minimization of conventional immunosuppressive therapy. The clinical course of each trial participant is shown in a time-to-exit analysis, with each patient's indication for withdrawal from the study protocol. (c) Patient KGW exited the trial in the 12th week post-transplantation owing to clinical suspicion of acute rejection. A biopsy taken at this time showed no evidence of a rejection process. (d) Prior to reducing patient GM's treatment with tacrolimus to sub-therapeutic levels at week 24, a graft biopsy showed no signs of rejection.

© 2008 The Authors
Journal compilation © 2008 European Society for Organ Transplantation

Participants in the TAIC-I study all received their first renal transplant, each from deceased donors. In every case, surgery proceeded without complications and the early renal function of all recipients was satisfactory, with the exception of patients FK and UF (see below). Initially, patients received a conventional triple immunosuppres-

(a)

Trial protocol

■ Administration of TAICs

Immunosuppression with Tacrolimus, Sirolimus and corticosteroids

Steroid weaning phase

Sirolimus weaning phase

Minimisation of Tacrolimus treatment (8–10ng/ml)

Further reduction of Tacrolimus therapy

Monitoring phase

(b)

WW Excluded

FK Excluded

DW Exit: Day 28, DVT

UF Exit: Day 39, Rejection – Banff Grade III

MR Exit: Day 53, Unconfirmed rejection episode

ME Exit: Day 54, Unconfirmed rejection episode

MT Exit: Day 58, Rejection – Banff Grade II

GH Exit: Day 63, Rejection – Banff Grade I

KGW Exit: Day 84, Unconfirmed rejection episode

UH Exit: Day 164, Withdrew consent

GS Exit: Day 168, Withdrew consent

GM Exit: Day 193, Rejection – Banff Grade I

0 4 8 12 16 20 24 28 32

Time post-transplantation (weeks)

Legend

Exit from the trial with no evidence of rejection

Exit from the trial with a clinical suspicion of rejection, but without histological confirmation

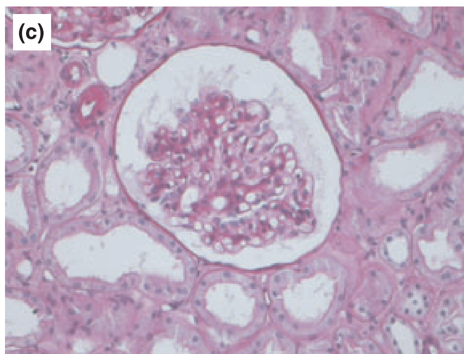
Exit from trial with histologically confirmed rejection

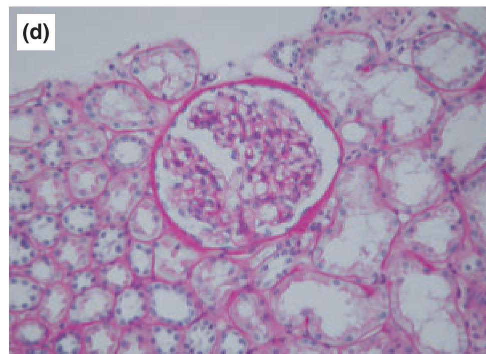
Complete withdrawal of all immunosuppressive therapy

Sub-therapeutic Tacrolimus monotherapy, trough serum levels: ≤4.1ng/ml

Tacrolimus monotherapy, trough serum levels: ≥5–8 ng/ml

Tacrolimus monotherapy, trough serum levels: ≥8–10 ng/ml





© 2008 The Authors

sive regimen comprising tacrolimus, sirolimus and glucocorticoids. TAICs were prepared from donor splenocytes and administered to the recipient on the fifth day postoperatively by central venous infusion (Table 2). WW was excluded from the trial because only 5.5×10^5 TAICs per kg bodyweight (BW) were available for transfusion, whereas the remaining patients each received in excess of 1×10^6 TAICs per kg BW. No acute adverse effects of TAIC administration were observed.

One patient, FK, showed evidence of an early rejection episode prior to TAIC infusion, with an abnormally elevated serum creatinine and histological evidence of rejection on day 5. Although the patient was subsequently infused with 1.9×10^6 TAICs per kg BW, he was excluded from further participation in the trial.

Phased withdrawal of steroid immunosuppression

On the 28th day post-transplantation, the graft function of nine trial participants met the stipulated conditions for steroid tapering. DW continued to receive steroid therapy because he had moderately elevated serum creatinine values in the second week postoperatively, which increased by >25% in the third week. A rejection episode was suspected, but a graft biopsy did not confirm this diagnosis and the episode resolved without intervention after 7 days; the transient deterioration in graft function was subsequently attributed to a urinary tract infection. This same patient then developed a right leg DVT and was consequently withdrawn from the trial. A second patient, ME, was maintained on 5 mg prednisolone OD after week 4. ME defaulted from the trial in week 7 with suspected acute rejection, although this could not be histologically verified.

Steroids were tapered over 14 days (during the fifth and sixth weeks post-transplantation) without adverse consequence in seven of the eight patients. UF defaulted from the trial in week 5 with severe, acute rejection. Throughout his participation in the study, UF had registered marginal graft function, with slow initial urine production and persistently elevated creatinine levels in the 2 weeks prior to steroid weaning. Examination of a biopsy from day 28 revealed Banff Grade III rejection.

Phased withdrawal of sirolimus and tacrolimus immunosuppression

At the end of the sixth week, seven patients met the criteria for sirolimus cessation and six of these tolerated complete withdrawal. MR exited the study during the period of sirolimus minimization with increased serum creatinine levels. Biopsies failed to confirm the diagnosis of an acute rejection episode, but renal function was restored following treatment with bolus steroids.

The remaining six patients qualified for minimization of tacrolimus therapy during weeks 9–12. As tacrolimus treatment was being reduced, two patients underwent rejection. MT first recorded a rise in serum creatinine in week 8, when tacrolimus had been reduced to 5 mg OD; at this time, a graft biopsy revealed Banff Grade II rejection. GH suffered a rejection episode in week 9, with a biopsy showing Grade I rejection. In the 12th week postoperatively, KGW experienced an acute rise in serum creatinine. This episode was treated as acute rejection, but biopsies did not support this clinical diagnosis (Fig. 4c). The patient thus exited the trial protocol on day 79, having otherwise tolerated tacrolimus monotherapy for 22 days.

Monitoring of transplant recipients after withdrawal from immunosuppression

Of the 12 patients enrolled in TAIC-I, three patients completed the programme of immunosuppressant minimization. Two patients, GS and UH, elected to discontinue their involvement in the study, exiting on days 168 and 164 respectively. The participation of UH in the trial was uncomplicated: he was withdrawn from prednisolone and sirolimus treatment within 43 days of transplantation, and his immunosuppression was further reduced to 3 mg tacrolimus OD by day 136 without adverse effect. GS had a stable serum creatinine, except for a rise in week 5 attributed to calcineurin inhibitor toxicity, which resolved within 3 days when tacrolimus treatment was lowered from 9 to 3 mg daily. A graft biopsy in week 6 showed no signs of rejection. When patient GS retracted her consent, immunosuppression had been reduced to 1 mg tacrolimus OD for a period of 21 days with no apparent adverse effect.

GM remained within the study until day 188 postoperatively. This patient had normal graft histology at week 24 (Fig. 4d) and registered normal, stable serum creatinine levels until week 28, when a significant increase was observed. Duly, a graft biopsy from day 188 was reported as showing a Banff Grade I rejection response. At the time of the rejection, the patient had taken no immunosuppressive therapy for 6 days, and prior to this had only received tacrolimus doses of 0 and 2 mg on alternating days for 2 weeks. The rejection episode was treated with bolus steroids and had resolved within 10 days.

Synopsis of patient outcomes

Of the 12 patients enrolled in TAIC-I, one was withdrawn because too few cells were administered and a second was

© 2008 The Authors

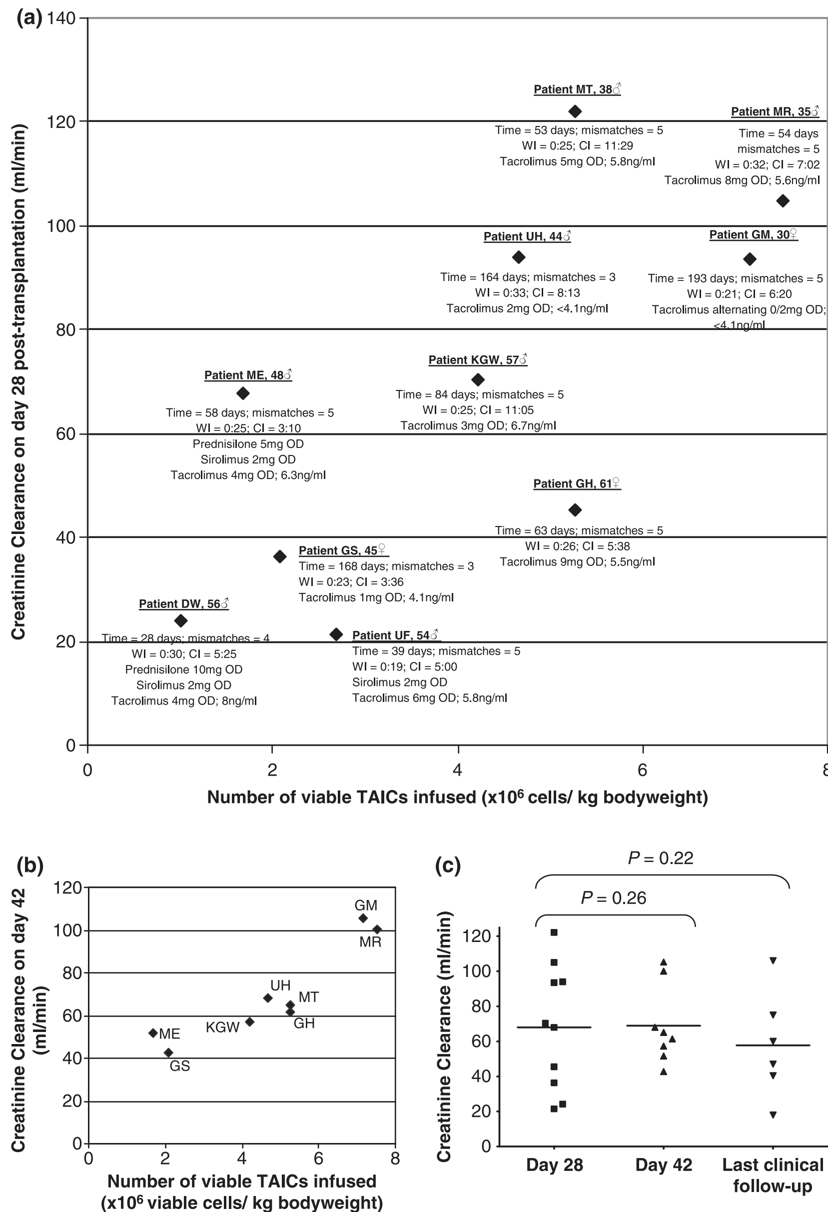


Figure 5 (a) A positive correlation exists between CL_{Cr} on day 28 and the dose of TAICs administered per kg of bodyweight (R^2 = 0.53). This correlation cannot be wholly attributed to the co-dependence of bodyweight-adjusted TAIC dosage and CL_{Cr} at day 28 on the mass of the patient, as the correlation between these variables is weak (R^2 = 0.16). Similarly, the anticipated relationship between tacrolimus dose and CL_{Cr} does not account for the observed relationship between TAIC dosage and CL_{Cr}. The minimal doses of immunosuppressants tolerated by each patient are given: tacrolimus doses (mg) and trough serum tacrolimus levels (ng/ml) are determined. (b) Amongst the eight patients remaining within the trial on day 42, the correlation between CL_{Cr} and TAIC dose was more pronounced (R^2 = 0.82). (c) There was no significant decrease in CL_{Cr} between day 28 and day 42, nor was CL_{Cr} significantly different between day 28 and the last clinic follow-up.

excluded on day 6 on account of an acute rejection episode, which began before TAIC infusion. Neither case provides any evidence concerning the safety of TAICs and must be disregarded in the analysis. Four patients experienced a confirmed rejection episode as conventional immunosuppression was withdrawn. In all cases renal function was restored by reintroduction of conventional immunosuppressive treatment and none of the trial participants required repeat transplantation or dialysis within the trial period. A further four patients each registered

© 2008 The Authors

acute rises in serum creatinine levels, which were necessarily treated as rejection episodes, but which could not be retrospectively confirmed as such by histology. The remaining two patients withdrew from the trial with no suspicion of rejection, having been successfully weaned to tacrolimus monotherapy with trough serum levels of <4 ng/ml.

Figure 5a relates patient outcomes (in terms of CL_{Cr} on day 28, minimal tolerated immunosuppression and time-to-exit) to the dose of TAICs received by individual patients, ischaemia times and the number of HLA mismatches. Representing the trial data in this way illuminates an interesting trend: there is a positive correlation between the bodyweight-adjusted dose of TAICs administered to patients and their CL_{Cr} at day 28. Among the eight patients remaining within the trial, the correlation was more pronounced on day 42 (Fig. 5b). This apparent relationship must be treated with caution because the sample size is small.

With the exception of DW, who exited the trial before steroid weaning, the graft function of all the TAIC-I participants was satisfactory at their last clinic follow-up; the clinical status of the patients is summarized in Table 3. Figure 5c compares the patients' CL_{Cr} on day 28 and day 42, and at the last clinic attendance (36 ± 8 months). Four of six patients recorded a lower CL_{Cr} on day 28 than at their last follow-up, but the mean reduction of 14.1 ml/min was not significant. This reduction is consistent with previously reported outcomes in similar cohorts of conventionally treated renal transplant recipients [28].

Discussion

The TAIC-I study has demonstrated that it is possible to produce clinical-grade TAICs from splenic mononuclear cells of deceased donors and to subsequently infuse these cells into renal transplant recipients without acute adverse consequences. More specifically, no harm was caused by the administration of quite large numbers of cells (up to 5×10^8 viable cells) via a central line, with no evidence of embolism, transfusion reactions or introduction of infection. There was no evidence of graft-versus-host reactions caused by the TAICs or contaminating lymphocyte populations. Furthermore, there was no evidence that TAICs sensitized the recipients to graft antigens or that the cells themselves can otherwise accelerate rejection. To date, none of the TAIC-I participants have experienced any delayed complications from TAIC infusion, although the mean follow-up time is now only 36 months. Thus, it is concluded that the infusion of TAICs is a practicable and safe clinical procedure in the acute and medium term.

From this report, the limitations of the TAIC-I trial are very clear: No strong conclusions about the possible beneficial effects of TAIC treatment can be drawn from such a small study cohort and, without a relevant control arm, it is not possible to attribute any therapeutic effect to the cell infusion. Moreover, as the TAIC-I study aimed to reduce the immunosuppressive therapy of all participants equally, it is clear that patients who were able to tolerate significant reductions in their immunosuppression registered poor outcomes because their stable, low-dose immunosuppression was further minimized; for this reason, comparison with published studies of immunosuppressant minimization is problematic [19–21, 29–33]. Whether TAIC therapy confers any additional benefit in safely establishing renal transplant recipients on low-dose tacrolimus monotherapy, beyond that already achieved with other clinical protocols, must be assessed in future clinical studies.

Closer monitoring of TAIC-treated patients, with greater reliance on special indices of imminent rejection episodes, should allow a safer titration of conventional immunosuppression. With this mind, a second trial of TAIC therapy has been initiated (the TAIC-II study) as a subproject of the RISET consortium, in which TAICs are being given to recipients of renal transplants from living donors as part of an immunosuppressant dose-reduction regimen [34].

Currently, we do not know the most effective TAIC dose. In the TAIC-I study, an average of 3.95×10^6 TAICs per kg bodyweight were infused, but compared to the number of TAIC administered in murine models (5×10^6 cells per animal, itself an arbitrary number) the number of cells given to patients was substantially lower [35]. In this context, the apparent trend observed in those patients who received greater TAIC numbers to have greater creatinine clearances on days 28 and day 42 is interesting. Unfortunately, it is technically difficult to produce more TAICs from a single donor spleen. In the TAIC-II study, cells from leukapheresis products are being used instead of splenic mononuclear cells to generate TAICs, so patients enrolled in TAIC-II have received substantially more cells [34]. One benefit of using TAICs from living organ donors is that repeated TAIC treatments are possible, both before and after transplantation. We suggest that successive rounds of TAIC administration may be the optimal way of delivering large numbers of cells to patients.

The optimal timing of TAIC administration with respect to transplantation is not known. Using rat allogeneic heart and kidney transplant models, it has been shown that treatment with TAICs on the fifth day preoperatively indefinitely prolongs graft survival (F. Fändrich, unpublished data), whereas the cells only afford a slight prolongation of graft survival when delivered postoperatively (S. Inoue *et al.* Abstract #1042, American Transplant Congress 2006, Boston, MA). Both from a clinical

© 2008 The Authors

Table 3. Clinical status of the TAIC-I participants at their last clinic attendance.

		Clinical status at the last follow-up			
Patient	CL_{Cr} on day 28 (ml/min)	Months postoperatively	Creatinine (mg/dl)	CL_{Cr} (ml/min)	Maintenance therapy
DW	24.1	39	2.30	NA	1 mg Tacrolimus BD 500 mg Mycophenolate BD 5 mg Prednisolone OD
UF	21.4	32	3.66	18.0	1.5 mg Tacrolimus BD 1 mg Rapamycin OD 5 mg Prednisolone OD
MR	105.0	47	1.30	75.0	2 mg Tacrolimus BD
ME	67.9	46	2.56	40.4	1 mg Tacrolimus BD 5 mg Prednisolone OD
MT	122.1	25	1.12	NA	3 mg Tacrolimus BD 4 mg Rapamycin OD
GH	45.4	27	1.20	47.0	3 mg Tacrolimus BD 4 mg Prednisolone OD
KGW	70.3	36	1.1	60.0	2 mg Tacrolimus BD 2 mg Rapamycin OD 5 mg Prednisolone OD
UH	93.9	26	1.30	NA	1.5 mg Tacrolimus BD
GS	36.3	40	1.80	NA	1.5 mg Tacrolimus BD 250 mg Mycophenylate BD 5 mg Prednisolone OD
GM	93.4	44	0.79	106.0	2 mg Tacrolimus OM 1 mg Tacrolimus ON 2.5 mg Prednisolone OD

perspective and from our understanding of the development of tolerance to transplanted organs, we suppose it would be optimal to begin immune-conditioning with TAIC infusions prior to transplantation [28]. Whilst TAIC pretreatment is clearly impracticable in the case of transplantation from a deceased donors, the use of peripheral blood monocytes from living donors permits exactly this approach: In the TAIC-II study, patients are being treated with TAICs 5 days before transplantation [34].

By their mode of derivation and cell surface phenotype, TAICs from rats and mice are comparable to human TAICs. It has been asserted elsewhere that mouse TAICs are a subset of macrophages in a unique state of activation, distinct from previously described M1- and M2-polarized macrophage subsets and monocyte-derived DC [16]. Likewise, data from this study (and additional unpublished results, J. A. Hutchinson) indicate that human TAICs do not correspond with any previously described macrophage subset. It is beyond the scope of this paper to discuss the mechanisms by which human TAICs might exert a therapeutic effect; however, it is proposed that TAICs could act through recipient-derived T-cell suppressive T-cell subsets. We speculate that engrafted donor-derived TAICs polarize the T-cell response of the recipient towards tolerance of the graft alloantigens, perhaps by providing an appropriate co-stimulatory *niche* for recipient T-regulatory cells. This hypothesized mechanism of TAIC function has clear parallels with the postulated mechanisms underlying the therapeutic effect of donor-specific blood transfusion [36–38].

In conclusion, it can confidently be said that TAIC therapy is both safe and clinically feasible, although in the setting of transplantation from deceased donors, the need to infuse TAICs postoperatively is a tangible disadvantage. Importantly, the TAIC-I trial has highlighted several possible aspects of TAIC therapy which might be optimized to achieve clinically appreciable outcomes and these improvements are being implemented in the TAIC-II trial protocol.

Authorship

JAH: data analysis, wrote paper. PR: data analysis, edited manuscript. BGB-E: trial coordinator. MS: participating clinician. LR: participating clinician; UK: principal investigator. EKG: senior scientific participant, proof-reading. FF: senior scientific and clinical participant.

Acknowledgements

The authors would like to thank Professors Leslie Brent and Roy Calne for their very valuable contributions to the final preparation of this manuscript. The trial was supported by RISET (Reprogramming the Immune-Sys-

© 2008 The Authors

tem for the Establishment of Tolerance, a multinational project financed by the European Commission). This work is partly funded by DFG grant, number GE 1188/1-1.

References

1. Bluestone JA, Thomson AW, Shevach EM, Weiner HL. What does the future hold for cell-based tolerogenic therapy? *Nat Rev Immunol* 2007; **7**: 650.
2. Turnquist HR, Raimondi G, Zahorchak AF, Fischer RT, Wang Z, Thomson AW. Rapamycin-conditioned dendritic cells are poor stimulators of allogeneic $CD4^+$ T cells, but enrich for antigen-specific T regulatory cells and promote organ transplant tolerance. *J Immunol* 2007; **178**: 7018.
3. Lan YY, Wang Z, Raimondi G, *et al.* "Alternatively activated" dendritic cells preferentially secrete IL-10, expand $Foxp3^+$ $CD4^+$ T cells and induce long-term organ allograft survival in combination with CTLA4-Ig. *J Immunol* 2006; **177**: 5868.
4. Bériou G, Pêche H, Guillonneau C, Merieau E, Cuturi M. Donor-specific allograft tolerance by administration of recipient-derived immature dendritic cells and suboptimal immunosuppression. *Transplantation* 2005; **79**: 969.
5. Bonham CA, Peng L, Liang X, *et al.* Marked prolongation of cardiac allograft survival by dendritic cells genetically engineered with NF-κB oligodeoxyribonucleotide decoys and adenoviral vectors encoding CTLA4-Ig. *J Immunol* 2002; **169**: 3382.
6. Morelli AE, Thomson AW. Dendritic cells: regulators of alloimmunity and opportunities for tolerance induction. *Immunol Rev* 2003; **196**: 125.
7. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol* 2003; **21**: 685.
8. Jiang S, Lechler RI, He XS, Huang JF. Regulatory T cells and transplantation tolerance. *Hum Immunol* 2006; **67**: 765.
9. Thomson AW, Fairchild RL. The Last 5 years of basic science investigation in transplant immunology. *Am J Transplant* 2006; **6**: 1768.
10. St Clair EW, Turka LA, Saxon A, *et al.* New reagents on the horizon for immune tolerance. *Annu Rev Med* 2007; **58**: 329.
11. Lechler RI, Sykes M, Thomson AW, Turka LA. Organ transplantation: how much of the promise has been realized? *Nat Med* 2005; **11**: 605.
12. McCurry KR, Colvin BL, Zahorchak AF, Thomson AW. Regulatory dendritic cell therapy in organ transplantation. *Transpl Int* 2006; **19**: 525.
13. Rutella S, Lemoli RM. Regulatory T cells and tolerogenic dendritic cells: from basic biology to clinical applications. *Immunol Lett* 2004; **94**: 11.
14. Fändrich F, Lin X, Chai GX, *et al.* Preimplantation-stage stem cells induce allogeneic graft tolerance without supplementary host conditioning. *Nat Med* 2002; **8**: 171.
15. Fändrich F, Zhou X, Schlemminger M, Glass B, Lin X, Dreßke B. Future strategies for tolerance induction: a comparative study between hematopoietic stem cells and macrophages. *Hum Immunol* 2002; **63**: 805.
16. Brem-Exner BG, Sattler C, Hutchinson JA, *et al.* Macrophages driven to a novel state of activation have anti-inflammatory properties in mice. *J Immunol* 2008; **180**: 335.
17. Heumann A, Ungefroren H, Ruhnke M, *et al.* Charakterisierung nichtdendritischer monozytärer Zellen mit Immunregulatorischen Eigenschaften. Deutsche Gesellschaft für Chirurgie. *Chir Forum* 2005 [abstract]. 2005; **34**: 271.
18. Fändrich F. Induction of tolerance in clinical organ transplantation. *Nephrol Dial Transplant* 2006; **21**: 1170.
19. Coupes BM, Speake M, Johnson RWG, Roberts ISD, Campbell B, Short CD. Tacrolimus monotherapy in renal transplantation: four-year data. *Transplant Proc* 2002; **34**: 1612.
20. Shapiro R, Jordan ML, Basu A, *et al.* Kidney transplantation under a tolerogenic regimen of recipient pretreatment and low-dose postoperative immunosuppression with subsequent weaning. *Ann Surg* 2003; **238**: 520.
21. Starzl TE, Murase N, Abu-Elmagd K, *et al.* Tolerogenic immunosuppression for organ transplantation. *Lancet* 2003; **361**: 1502.
22. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 2005; **5**: 953.
23. Mantovani A, Sica A, Locati M. Macrophage polarization comes of age. *Immunity* 2005; **23**: 344.
24. Stout RD, Suttles J. Functional plasticity of macrophages: reversible adaptation to changing microenvironments. *J Leukoc Biol* 2004; **76**: 509.
25. Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J Exp Med* 1999; **189**: 1363.
26. Munn DH, Pressey J, Beall AC, Hudes R, Alderson MR. Selective activation-induced apoptosis of peripheral T cells imposed by macrophages. A potential mechanism of antigen-specific peripheral lymphocyte deletion. *J Immunol* 1996; **156**: 523.
27. Reis e Sousa C. Dendritic cells in a mature age. *Nat Rev Immunol* 2006; **6**: 476.
28. Renkens JJM, Rouflart MMJ, Christiaans MHL, van den Berg-Loonen EM, van Hooff JP, van Heurn LWE. Outcome of nonheart-beating donor kidney with prolonged delayed graft function after transplantation. *Am J Transplant* 2007; **5**: 2704.
29. Tan HP, Kaczorowski D, Basu A, *et al.* Steroid-free monotherapy after pretransplantation thymoglobulin or Campth and laparoscopy in living donor renal transplantation. *Transplant Proc* 2005; **37**: 4235.
30. Krämer BK, Krüger B, Mack M, *et al.* Steroid withdrawal or steroid avoidance in renal transplant recipients: focus on tacrolimus-based immunosuppressive regimens. *Transplant Proc* 2005; **37**: 1789.

© 2008 The Authors

31. Tan HP, Kaczorowski DJ, Basu A, *et al.* Living donor renal transplantation using alemtuzumab induction and tacrolimus monotherapy. *Am J Transplant* 2006; **6**: 2409.
32. Bäckman L, Åselius H, Lindberger K, *et al.* Steroid-free immunosuppression in kidney transplant recipients and prograf monotherapy: an interim analysis of a prospective multicenter trial. *Transplant Proc* 2006; **38**: 2654.
33. Thomas PG, Woodside KJ, Lappin JA, Vaidya S, Rajaraman S, Gugliuzza KK. Alemtuzumab (Campath 1H) induction with tacrolimus monotherapy is safe for high immunological risk renal transplantation. *Transplantation* 2007; **83**: 1509.
34. Hutchinson JA, Brem-Exner BG, Riquelme P, *et al.* A cell-based approach to the minimization immunosuppression in renal transplantation. *Transplant Int*: 10.1111/j.1432-2277.2008.00692.x [E-pub ahead of print].
35. Exner BG, Scherer MN, Koehl GE, *et al.* Hyperacute rejection of transplanted rat hearts is abrogated by the use of in vitro generated donor-derived transplant-acceptance-inducing cells (TAIC). *Transplantation* 2004; **78**(Suppl. 1): 530.
36. Burlingham WJ. The blood transfusion effect. In: Thiru S, Waldmann H, eds. *Pathology and Immunology of Transplantation and Rejection*. Oxford: Blackwell Science, 2001: 92–115.
37. Waanders MM, Roelen DL, Brand A, Claas FH. The putative mechanism of the immunomodulating effect of HLA-DR shared allogeneic blood transfusions on the alloimmune response. *Transfus Med Rev* 2005; **19**: 281.
38. Roelen D, Brand A, Claas FJ. Pretransplant blood transfusions revisited: a role for $CD4^+$ regulatory T cells? *Transplantation* 2004; **77**: S26.

© 2008 The Authors