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ORIGINAL ARTICLE

Postoperative intravenous infusion of donor-derived transplant acceptance-inducing cells as an adjunct immunosuppressive therapy in a porcine pulmonary allograft model

Gregor Warnecke,[1]* James A. Hutchinson,[2]* Paloma Riquelme,[2] Bianca Kruse,[1] Stefanie Thissen,[1] Murat Avsar,[1] Gregor Zehle,[2] Thomas Steinkamp,[1] Carsten Peters,[1] Rolf Baumann,[3] Felix Gövert,[2] Hendrik Ungefroren,[2] Florian Länger,[4] André R. Simon,[1] Johann H. Karstens,[3] Volkhard Kaever,[5] Axel Haverich,[1] Fred Fändrich[2] and Martin Strüber[1]

1 Division of Thoracic and Cardiovascular Surgery, Hannover Medical School, Hannover, Germany
2 Laboratory for Transplantation Research, Department of General and Cardiothoracic Surgery, University Hospital of Schleswig-Holstein, Kiel, Germany
3 Department of Radiotherapy and Special Oncology, Hannover Medical School, Hannover, Germany
4 Department of Pathology, Hannover Medical School, Hannover, Germany
5 Department of Pharmacology, Hannover Medical School, Hannover, Germany

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Correspondence
PD Dr med. Martin Strüber, Division of Thoracic and Cardiovascular Surgery, Hannover Medical School, Carl-Neuberg-Str. 1, Hannover 30623, Germany. Tel.: +49 511 532 6588; fax: +49 511 532 8446; e-mail: strueber.martin@mh-hannover.de

*These authors contributed equally to the preparation of this manuscript.

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Summary

There is very limited published information testifying to the safety and possible complications of cell-based therapies. Accurately assessing the potential risks of translating novel, cell-based immunosuppressive protocols into clinical trials is therefore extremely difficult. This report describes the use of a pulmonary allograft model in outbred miniature pigs as a preliminary step in the development of a safe, clinically feasible, cell-based immunosuppressive protocol. Single lung transplants were performed in 22 MHC Class I-mismatched donor–recipient pairs, which were randomized between four treatment groups. For the first 28 days postoperatively, all animals were immunosuppressed with methylprednisilone and tacrolimus, with or without preoperative irradiation; subsequently, pharmacological immunosuppression was stopped in all treatment groups. Animals in two groups also received a central venous infusion of donor-derived 'transplant acceptance-inducing cells' (TAICs) on the seventh and 14th days postoperatively. Allograft survival was monitored by sequential chest X-rays, bronchoscopies and transbronchial biopsy histologies. No acute adverse events were associated with the administration of TAICs and there was no evidence of accelerated graft rejection. The observations presented in this report represent an important first step towards the development of a clinically applicable protocol for the use of TAIC therapy in lung transplantation.

Introduction

There is growing interest in the possibility of establishing transplantation tolerance by adoptive transfer of tolerance-inducing cell types produced *ex vivo* [1–12]. If such strategies are ever to represent a clinically viable approach to transplant immunosuppression, it is immediately clear that the substrate cells must be readily obtained from donor or recipient, that these cells should be subjected to the least possible manipulation to minimize the rate of technical failure and cost, and that the therapeutic cells should be readily and effectively transferred into patients. These clinical considerations have underpinned research efforts in our laboratories and led to the identification of 'transplant acceptance-inducing cell' (TAIC) as a cell type which is particularly suitable for clinical use [1–4,13–17].

The TAIC is a type of immunoregulatory macrophage with the capacity to suppress mitogen-stimulated T-cell proliferative responses (Hutchinson JA et al. unpublished data). These cells can be readily identified by their characteristic morphology and cell-surface marker phenotype, which overlaps with, but does not correspond exactly to, that of previously described macrophage subsets. Human TAICs express higher levels of CD86 and HLA-DR than resting macrophages, but lower levels than classically activated macrophages, and are distinguished from M1- and M2-polarized macrophage subsets by the $CD14^{low/-}CD16^{-}CD32^{+}CD64^{+}$ $TLR2^{-}CD163^{low/-}CD206^{+}$ phenotype. Previous work has shown that acquisition of this phenotype depends upon adherence to tissue culture plastic and an undefined component of human serum.

Two Phase-I clinical safety trials of TAICs as an adjunct immunosuppressive therapy in renal transplantation have been undertaken: the TAIC-I and TAIC-II trials [1,2]. These trials demonstrated that TAIC administration is both safe and clinically feasible, and the clinical outcomes of patients in the TAIC-II trial suggested that preoperative TAIC administration can establish a donor-specific state of *prope* tolerance in the recipient [1–4]. Of course, there is an obvious difficulty in adapting the tolerance-inducing protocol used in living donor kidney transplantation to deceased donor lung transplantation, namely, that preoperative administration human TAICs is impractical. However, studies in a nonimmunosuppressed mouse heterotopic heart transplant model have demonstrated that TAIC administration on the fifth day postoperatively conferred a benefit in terms of allograft survival (S. Inoue *et al.* Abstract #1042, American Transplant Congress 2006, Boston, MA). Therefore, the objective of the current preclinical study was to assess the feasibility and safety of a treatment protocol involving the postoperative infusion of TAICs which might be applied in clinical lung transplantation.

The first part of this report describes the morphology and cell surface phenotype of porcine TAICs and draws comparisons with human TAICs. The second part describes a preliminary preclinical trial of postoperative administration of porcine TAICs as a tolerance-inducing therapy in Göttinger minipigs which received a left-sided pulmonary allograft. This study took the form of a prospective, randomized controlled trial. The primary objective of this study was to assess the safety of TAIC administration in a preclinical transplantation model. The secondary objective was to monitor graft survival in transplant recipients treated with TAICs as part of a clinically practicable immunosuppressive protocol.

Materials and methods

Animals

Forty-eight animals (aged 12–15 months) were selected from an outbred specific pathogen-free (SPF-) Göttinger minipig herd, consisting of eight distinct breeding lines (Ellegaard, Dalmose, Denmark). Animals from different breeding lines were tissue-typed prospectively using a lymphocytotoxic assay. Subsequently, donors and recipients were mismatched for the MHC class I DC 80 and H04 haplotypes [18] and for reactivity with the MHC class I haplotyped specific monoclonal antibody 74-11-10. All animals received humane care in compliance with the German animal protection legislation, the 'Principles of Laboratory Animal Care', and the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996.

Surgical technique

The surgical technique of left-sided single lung transplantation in pigs has been described elsewhere [19]. Briefly, lungs were harvested from donor animals (15–25 kg) after Euro-Collins cold flush perfusion. A permanent vascular access double-lumen 3.2 mm Quinton atrial catheter was inserted into the right jugular vein of recipient animals (15–25 kg). After thoracotomy in the fourth intercostal space the left lung was removed. The allogeneic lung was then transplanted using a telescoping bronchial anastomosis technique with running posterior wall and interrupted anterior wall 4-0 polydioxanone sutures. The venous atrial cuff and the pulmonary artery were anastomosed with running polypropylene sutures. After closure of the thorax and extubation, the animals were put in sterile wooden boxes and provided with heating lamps and drinking water.

Preparation of pig TAICs for cell therapy and phenotypic characterization

Transplant acceptance-inducing cells were generated from peripheral blood mononuclear cells (PBMC) of donor animals following a protocol adapted from Hutchinson *et al.* [1]. Briefly, heparinized blood was drawn from male donor animals at the time of lung harvest and mononuclear cells were isolated by gradient centrifugation (Ficoll-Paque; Amersham Pharmacia Biotech AB, Uppsala, Sweden). The cells were cultured for 5 days in RPMI 1640 medium containing 10% fetal calf serum (FCS; Gibco, Karlsruhe, Germany) and 5 ng/ml recombinant human M-CSF (R&D Systems, Wiesbaden-Nordenstadt,

Germany). After 1 and 3 days, cultures were gently washed to select for adherent cells and fresh medium was added to the adherent cell layer. On day 4, 25 ng/ml recombinant porcine interferon (IFN)-γ (R&D Systems) was added to the cultures for 16–20 h. The next day, the adherent cell fraction was harvested with a cell scraper, washed before use and infused intravenously on the seventh and 14th day postoperatively. Cell doses administered to each animal are given in Table 1. The numbers of viable cells given to each animal ranged between 1.1×10^6 and 13.0×10^6 because of both the number and quality of the substrate monocytes, which varied between donors, and variations in the *ex vivo* manipulation of the cells, particularly their harvesting. On-going technical refinements of the production and administration of human TAICs have been discussed elsewhere. In some experiments, porcine macrophages were generated in TAIC medium containing M-CSF, as described above, but either 10% human AB serum (hABS; Lonza, Verviers, Belgium) or 10% autologous pig serum (Ellegaard) was used instead of FCS.

Flow cytometry

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 (clone MIL-2; AbD Serotec, Oxford, UK), CD16 (clone G7; AbD Serotec), CD18 [Becton Dickinson (BD), #555924, Heidelberg, Germany]. 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 using Cell Quest software (BD).

Technique of irradiation and immunosuppression

Irradiation consisted of adjusted whole body irradiation (1.5 Gy total dose) and thymic irradiation in a cervico-

Table 1. Clinical outcomes of pulmonary allograft transplantation in minipigs.

Animal	Time of trial exit (days)	Clinical outcome	Histological grading	Chest X-ray score	SLA-haplotype mismatches	TAICs (×10^6 cells)
Group 1: Pharmacological immunosuppression alone						
P01	25	Cachexia	A0	1	1	
P02	83	Acute rejection	A3	3	1 or 2	
P03	93	Acute rejection	A3	3	1 or 2	
P04	154	Acute rejection	A4	2	2	
P05	188	Chronic rejection	A1	3	1	
P06	343	Chronic rejection	A1	3	2	
Group 2: Pharmacological immunosuppression plus irradiation						
P07	49	Septicaemia	A0	0	1 or 2	
P08	68	Acute rejection	A4	4	1 or 2	
P09	69	Acute rejection	A3	4	1 or 2	
P10	92	Acute rejection	A3	4	1 or 2	
P11	130	Acute rejection	A3	3	1 or 2	
P12	700	Alive	A0	0	1	
Group 3: Pharmacological immunosuppression plus postoperative TAIC administration						
P13	13	Septicaemia	A0	1	1 or 2	13.0
P14	16	Septicaemia	A0	2	1	8.0
P15	84	Acute rejection	A4	4	2	2.5
P16	99	Acute rejection	A4	3	1	1.1
P17	131	Acute rejection	A3	4	2	2.0
P18	727	Elective sacrifice	A0	0	1 or 2	2.5
Group 4: Pharmacological immunosuppression plus irradiation and postoperative TAIC administration						
P19	65	Anaemia	A0	3	1 or 2	1.4
P20	97	Acute rejection	A2	3	1 or 2	5.0
P21	100	Acute rejection	A2	4	2	NA
P22	307	Elective sacrifice	A1	2	1	3.0
P23	342	Acute rejection	A4	4	1	NA
P24	1199	Elective sacrifice	A0	2	1 or 2	13.0

NA, data not available.

sternal field of 6 cm width and 12 cm length (7.0 Gy total dose) using a linear accelerator (Mevatron, Siemens, Germany) within 12 h before lung transplantation.

Intravenous pharmacological immunosuppression included 1.5 mg/kg/day methylprednisolone and tacrolimus (Fujisawa, Osaka, Japan) adjusted to blood trough levels of 16–26 ng/ml. Tacrolimus blood trough levels were monitored daily using a radioimmunoassay. Empiric intravenous antibiotic therapy consisted of 200 mg/day Ciprofloxacin (Bayer, Ludwigshafen, Germany). All immunosuppressive therapy was withdrawn on the 27th day postoperatively.

Experimental groups

The recipients were assigned to four different treatment groups (Table 1). All animals received a lung allograft, as described above. Animals in Group 1 received only pharmacological immunosuppression with tacrolimus ($n = 6$); animals in Group 2, were treated with tacrolimus and preoperative radiation ($n = 6$); animals in Group 3 were immunosuppressed with tacrolimus and received an intravenous infusion of donor-derived TAICs on day 7 and 14 postoperatively ($n = 6$); and animals in Group 4 received TAICs on days 7 and 14 postoperatively, as well as tacrolimus therapy and preoperative irradiation ($n = 6$). The trial protocol is shown schematically in Fig. 1.

Rejection monitoring

Sequential chest radiographs were performed and a score from 0 (no pathological changes) to 4 (homogenous infiltration of the left lung, normal right lung) was assigned by a reviewer who worked blindly. The bronchoscopic appearance of the bronchial mucosa was evaluated and broncho-alveolar lavages and transbronchial biopsies (TBB) were obtained. TBB sections were stained with hematoxylin–eosin and reviewed blindly. Histological acute and chronic rejection was graded referring to the ISHLT guidelines ranging from A0 to A4 and from B0 to B4, respectively [20]. Rejection was defined as a strictly left-sided infiltrate on the chest radiograph scored 3 or higher, together with a grade A2–A4 or B3–B4 histological rejection in the absence of infection.

Detection of leucocyte chimerism after TAIC therapy

The quantitative PCR assay used for chimerism analysis in the peripheral blood was based on the detection of the swine male specific repeat (MSR) DNA present on the Y-chromosome [21]. Identical methods, except for the target, have been used in rodents [22–24]. Briefly, blood DNA from samples taken before and 1 h after macrophage transplantation was extracted using the DNeasy Tissue Kit (Qiagen, Hilden, Germany). Genomic DNA was amplified with MSR or control primers listed below, with a product length of 185 bp for MSR primers, and 184 bp for control primers. Cycling was performed on an iCycler (Biorad, Hercules, CA, USA). SybrGreen was used for signal induction. Fluorescence was measured every two cycles and relative quantifications were made using a standard curve of 100%, 50%, 20%, 10%, 1%, 10^{-1}%, 10^{-2}%, 10^{-3}% male DNA diluted in DNA elution buffer AE (Qiagen) to calculate the percentage contingent of Y-chromosomal DNA. The quality as well as the total amount of pig DNA was assessed by a control PCR with pig S100C primers. The absolute number of Y-chromosomal PBMC was calculated as described above. Primers used were MSR upper 5′-CCA TCG GCC ATT GTT TTC CTG TTC A-3′, MSR lower 5′-CCT CTG TGC CCA CCT GCT CTC TAC A-3′, S100C upper 5′-ATG CTG GAA GGG ACG GTA ACA ACA-3′ and S100C lower 5′-GCT CAG CTG CTG TCT TTC ACT CGT-3′.

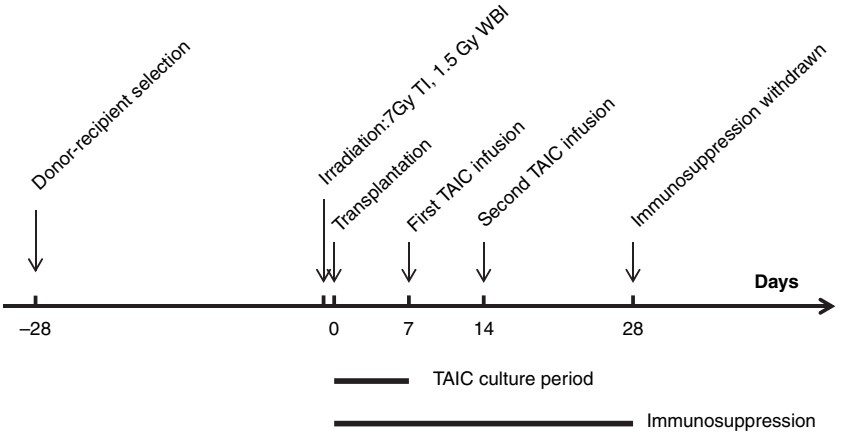

Figure 1 Treatment protocol for the preclinical safety trial of TAICs in pulmonary transplantation.

Statistical methods

Graft survival was compared between groups by calculation of Kaplan–Meier survival curves (Graph Pad Software Inc., San Diego, CA, USA). Animals which defaulted from the trial for reasons other than acute or chronic rejection were censored from the analysis. Spearman's correlation coefficients for nonparametric data were calculated to show or rule out influences of cumulative drug levels (areas under the curve) on graft survival using two-tailed testing.

Results

Morphology and marker phenotyping of porcine TAICs

The appearance and cell surface phenotype of macrophages in culture depends upon many factors, including cell plating density, the nature of tissue culture plastic and the type of serum to which the cells are exposed. Porcine macrophages were prepared using medium supplemented with either 10% autologous swine serum, 10% FCS or 10% hABS. Grown in pig serum, the resultant macrophages acquired a flattened morphology, not dissimilar to that previously described for human TAICs (Fig. 2a) except that these cultures contained numerous giant, multinucleated cells, which were presumably the result of cell fusion. In contrast, cells cultured in FCS adopted morphology similar to that of mouse IFN-γ-MdCs or resting human macrophages [15]. Porcine macrophages produced in hABS-containing medium most closely resembled human TAICs: the cells were roughly circular owing to a flattened 'skirt' of cytoplasm surrounding a central body of granular cytoplasm and were predominantly mononuclear.

The lack of antibodies against porcine macrophage markers presents significant difficulties in establishing the equivalence of human and porcine TAICs. Porcine macrophages grown under TAIC culture conditions in medium containing pig serum, FCS or hABS were analysed by flow cytometry for CD14 and CD16 expression, and CD18 was used as a positive control (Fig. 2b). No notable difference between the cells grown under different conditions was observed: each population was $CD14^+$, $CD16^+$ and $CD18^+$.

Porcine TAICs administered postoperatively did not significantly prolong pulmonary allograft survival

Twenty-two animals received pulmonary allografts and were then randomized to one of four immunosuppressive treatment groups. The six animals in Group 1 were treated with tacrolimus monotherapy for the first 28 days post-transplantation and received no immunosuppression subsequently (Fig. 3a). Of this group, one animal (P01) had to be killed on day 25 postoperatively owing to cachexia without macroscopic or histological evidence of rejection and must excluded from the trial analysis. The remaining five animals had normal bronchoscopic and radiological findings until the end of the period of immunosuppression (Figs 3b and 4). Subsequently, three of these animals (P02, P03 and P04) developed severe, radiologically evident infiltration of the left lung consistent with acute rejection and were accordingly killed on days 83, 93 and 154, respectively; histological examination

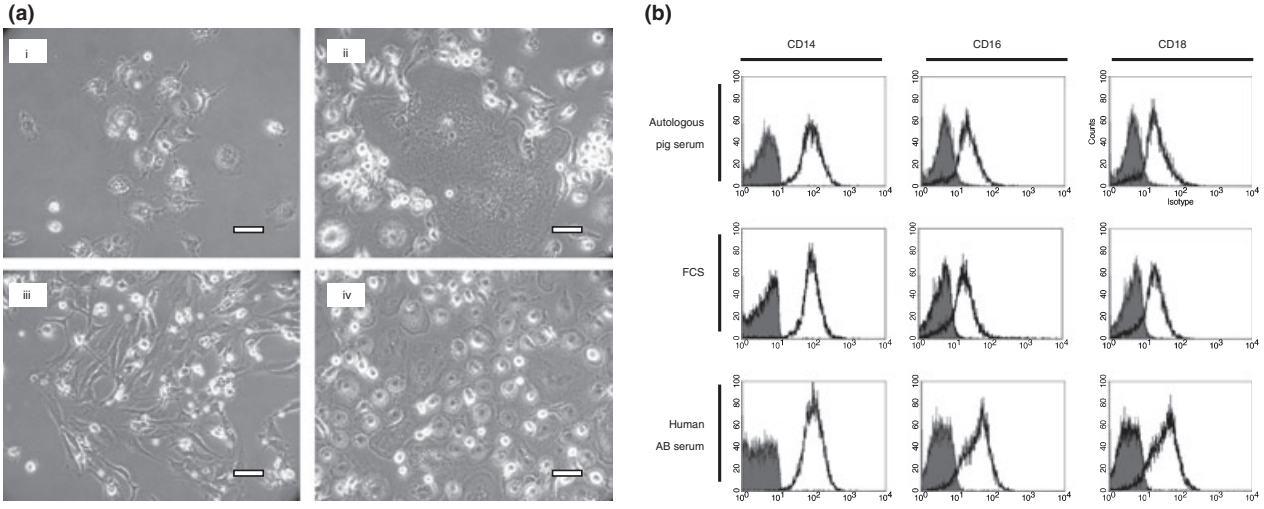

Figure 2 Morphology and immunophenotype of TAICs derived from peripheral blood monocytes of Göttinger minipigs. Representative data from one animal are shown. (a) Porcine macrophages were cultured in M-CSF-containing medium supplemented with (i, ii) autologous pig serum, (iii) FCS, or (iv) hABS. The resultant cells were strikingly different in appearance, with those grown in human serum most closely resembling human TAICs. Bar = 50 μm. (b) Expression of CD14, CD16 and CD18 by porcine macrophages.

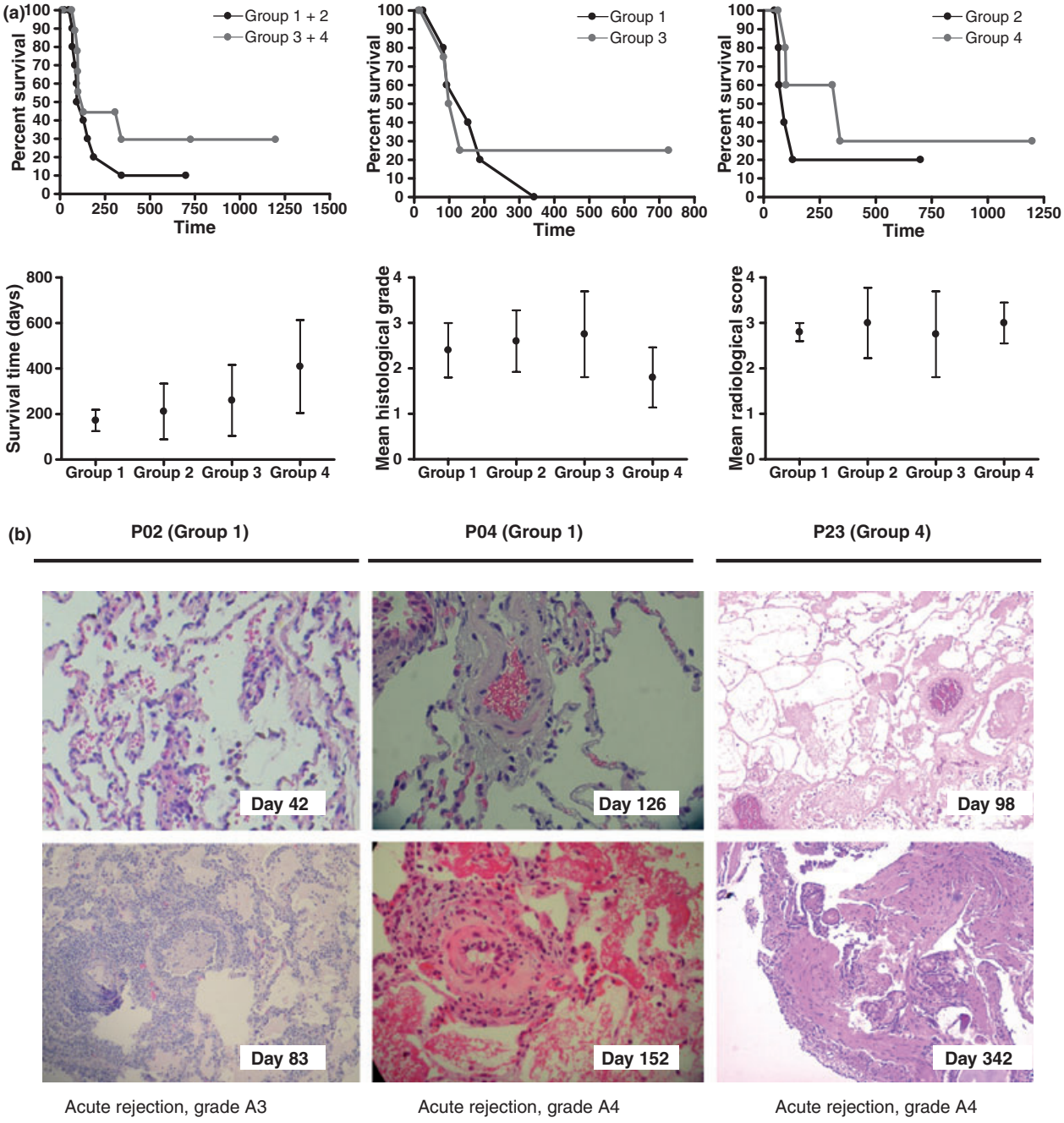

Figure 3 Outcomes in the preclinical safety study of postoperative TAIC administration in a minipig model of pulmonary transplantation. Animals were randomized to one of four treatment groups: Group 1 – pharmacological immunosuppression alone; Group 2 – pharmacological immunosuppression plus preoperative irradiation; Group 3 – pharmacological immunosuppression plus TAIC treatment; Group 4 – pharmacological immunosuppression, preoperative irradiation and postoperative TAIC treatment. (a) Allograft survival times, histological scores and radiologic appearances revealed no significant differences between the four treatment groups. (b) Representative histologies from three animals that underwent rejection episodes.

revealed perivascular mononuclear infiltrates of the transplanted organs graded A3/A4. The two other animals (P05 and P06) underwent rejection on days 188 and 343 respectively, and were duly killed. Excluding animal P01, the median allograft survival time in Group 1 was 154 days ($n = 5$).

No significant prolongation of transplant survival was observed in Group 2, which received a combination of

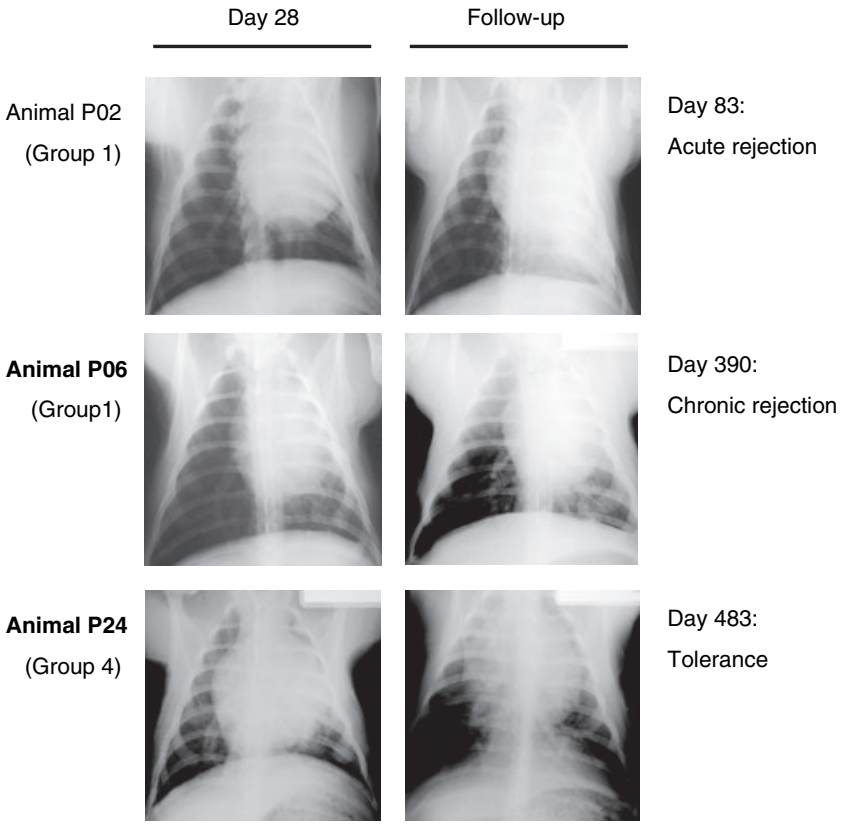

Figure 4 Representative chest X-rays from animals which received left-sided pulmonary allografts.

tacrolimus and preoperative irradiation: Excluding animal P07, which succumbed to septicaemia on day 49 postoperatively with no radiological or histological evidence of rejection, median graft survival time in Group 2 was 92 days ($n = 5$). Four animals (P08, P09, P10 and P11) showed signs of early, acute rejection and exited the trial on days 68, 69, 92 and 132, respectively. Animal P12 remained well on the 757 day postoperatively with no signs of rejection in chest radiographs or in a transbronchial biopsy.

In Group 3, which was treated with tacrolimus and intravenous infusion of TAICs on day 7 postoperatively, the median graft survival time was 115 days ($n = 4$), excluding animals P13 and P14 which developed septicaemia on days 13 and 16. The numbers of viable TAICs administered to each individual animal are given in Table 1. Three animals (P15, P16 and P17) underwent acute rejections on days 84, 99 and 131, respectively. The remaining animal (P18) was killed on day 727 postoperatively, at which time there was no histological evidence of rejection. Therefore, postoperative treatment with TAICs alone did not significantly prolong allograft survival time.

A total of six animals were treated with preoperative irradiation, one of which (P19) was killed on day 65 owing to severe anaemia, but showed no signs of rejection. Two further animals (P20 and 21) developed acute rejection episodes on days 97 and 100. The three remaining animals maintained functioning grafts beyond 178 days: Animals P22 and P23 developed very late progressive infiltrates of the grafted organ (days 307 and 342, respectively) and histological examination revealed rejection. Animal P24 was sacrificed on day 1199 postoperatively and postmortem examination showed signs of chronic rejection. Thus, excluding the two animals that defaulted from the study without having undergone rejection, the median graft survival time of 307 days was longer than in Group 2, but did not achieve statistical significance.

In a comparison of allograft survival time made between the TAIC-treated animals (Groups 3 and 4) and those which received no TAICs (Groups 1 and 2), no significant prolongation was evident. Similarly, there were no significant differences in histological and radiological scores between the TAIC-treated and untreated groups (Fig. 3).

TAIC administration did not result in established chimerism in the peripheral blood

Detection of male donor-derived leucocytes in the peripheral blood of female recipient animals was based on the detection of cells with a Y chromosome by PCR. Chimerism peaked within 24 h of pulmonary transplantation and decreased continuously thereafter in all groups (data not

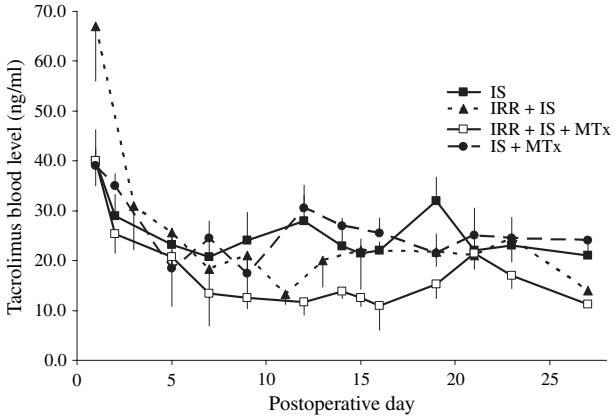

Figure 5 Whole blood trough levels of tacrolimus (ng/ml) during the first 28 days of the preclinical safety study. Drug doses were adjusted to blood levels daily. Values are mean ± SE.

shown). Samples taken 1 h after macrophage infusion did not reveal an additional increase of donor cell chimerism.

Tacrolimus whole blood trough levels did not correlate with allograft survival

Tacrolimus blood trough levels of 16–26 ng/ml were maintained throughout the first 27 days, which corresponded to maintenance doses in the range of 0.05 mg/kg BD (Fig. 5). Notably, tacrolimus trough levels were lowest in Group 4, so the apparent prolongation of graft survival in this group cannot be attributed to higher initial tacrolimus dosing [25]. The area under the curve of tacrolimus blood levels of individual animals did not correlate with allograft survival (data not shown). Serum urea and creatinine levels remained stable without differences in all animals (data not shown).

Discussion

Earlier work from our group established a preclinical single lung transplantation model in miniature swine, which was developed for testing novel immunosuppressive protocols [26,27]. The objective of this study was to design a protocol using TAICs that could be translated into a clinically feasible therapeutic approach in lung transplantation, taking into account that the identities of lung donors are only known a few hours ahead of transplant surgery. Hence, TAICs were manufactured from monocytes obtained at time of lung retrieval and the first infusion of the cultured macrophages was postponed to the seventh day postoperatively. In an attempt to mitigate the effect of delayed TAIC infusion, a group of recipient animals were treated with low-dose preoperative radiation, a technique used successfully in the experimental model previously [26].

The postoperative administration of TAICs by intravenous infusion was without acute adverse effects and TAICs did not cause an accelerated graft rejection. Importantly, although the cellular product contained some contaminating donor-derived T cells, there was no evidence of graft-versus-host disease. These observations are entirely consistent with safety data obtained in human patients which received pre- or postoperative infusions of TAICs as an adjunct immune-conditioning therapy in the renal transplantation setting [1,2].

Postoperative infusion of TAICs conferred no significant graft-protective effect. In interpreting this observation, it is important bear several important limitations of this study in mind. Firstly, the optimal dose of TAICs has not been established: in this study, cell doses in the range of 5×10^4 to 6×10^5 viable cells/kg were administered, whereas 10-fold more were used in the human renal transplantation studies (mean of 4×10^6 viable cells/kg) and an approximately 500-fold higher dose of 5×10^6 cells per animal was used for transplantation studies in rats and mice. Given that, in the human renal transplantation studies, a trend towards better renal graft function was seen with increasing cell doses, the relatively small numbers of TAICs used in this study may simply have been too low to achieve an observable effect [1]. Secondly, the recipient animals were deliberately treated with relatively high doses of tacrolimus (16–26 ng/ml blood trough levels) during the first 28 days of the study to prevent early rejection episodes. It is now generally recognized that high-dose calcineurin inhibitor treatment is suppressive of regulatory immune responses and we speculate that TAICs might exert a greater tolerogenic effect when administered with alternative, calcineurin inhibitor-minimizing immunosuppressive regimes. With specific regard to TAICs, it has been shown that both human TAICs and mouse IFN-γ-MdCs eliminate co-cultured T cells in an activation-dependent manner and it is thought that calcineurin inhibitors might affect this activity [15].

Perhaps the greatest obstacle in extrapolating from this study in minipigs to the clinical situation is that the equivalence of human TAICs and the porcine cells used in the transplantation studies has not been adequately established. This is not a trivial matter because macrophages are very plastic in their phenotype and it is unsound to base an interspecies comparison on only a few markers of unknown functional significance [28,29]. It has previously been demonstrated that the human TAIC phenotype only arises when the cells are prepared in medium supplemented with human serum and not FCS. Thus, the porcine TAICs used in the pulmonary transplantation may be more comparable with those human macrophages described by Munn *et al.*, or with

the mouse IFNγ-MdC described by Brem-Exner *et al.*, than with human TAICs [15,30,31]. In this study, porcine cells generated in FCS-containing medium were used because, by their mode of derivation, these cells were believed to be equivalent to the rat TAIC or mouse IFN-γ-MdC; however, further studies are planned using TAICs cultured with hABS, as these cells appear to be more similar to human TAICs in certain respects.

Given the nature of the TAIC, it is perhaps not surprising that attempts to detect circulating donor-derived cells after the cell infusion were unsuccessful. Human and pig TAICs in culture adhered tightly to plastic and have the cell-surface phenotype of matured macrophages, which are not usually found in peripheral blood. Mouse IFN-γ-MdCs distribute widely through noninflamed tissues and enter peripheral lymphoid tissues [15]. In a single case study, tracking of ^{111}In-labelled human TAICs infused preoperatively into a kidney transplant recipient showed that, over the course of 30 h, TAICs infused intravenously transited the lungs and migrated to the liver, spleen and haematopoietically active bone marrow (J.A. Hutchinson, unpublished data). Further studies of the trafficking of TAICs will be greatly facilitated by the development of the porcine pulmonary transplant model.

Authorship

JH: performed experiments, data analysis, wrote manuscript. GW: performed experiments, wrote manuscript. PR: performed experiments, data analysis. BK, ST, MA, GZ, TS, CP, RB, FG, HU, FL, ARS, JHK, VK: performed experiments. AH, FF, MS: principal investigators.

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Declaration

Professor Fändrich is board member of a company, Blasticon GmbH, which holds the intellectual property rights to TAICs.

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References

1. Hutchinson JA, Riquelme P, Brem-Exner BG, *et al.* Transplant acceptance-inducing cells as an immune-conditioning therapy in renal transplantation. *Transpl Int* 2008; **21**: 728.
2. Hutchinson JA, Brem-Exner BG, Riquelme P, *et al.* A cell-based approach to the minimization of immunosuppression in renal transplantation. *Transpl Int* 2008; **21**: 742.
3. Hutchinson JA, Roelen D, Riquelme P, *et al.* Preoperative treatment of a presensitized kidney transplant recipient with donor-derived transplant acceptance-inducing cells. *Transpl Int* 2008; **21**(8): 808.
4. Chatenoud L. The long and winding road towards induction of allograft tolerance in the clinic. *Transpl Int* 2008; **21**: 725.
5. Bluestone JA, Thomson AW, Shevach EM, Weiner HL. What does the future hold for cell-based tolerogenic therapy? *Nat Rev Immunol* 2007; **7**: 650.
6. St Clair EW, Turka LA, Saxon A, *et al.* New reagents on the horizon for immune tolerance. *Annu Rev Med* 2007; **58**: 329.
7. McCurry KR, Colvin BL, Zahorchak AF, Thomson AW. Regulatory dendritic cell therapy in organ transplantation. *Transpl Int* 2006; **19**: 525.
8. Lechler RI, Sykes M, Thomson AW, Turka LA. Organ transplantation: How much of the promise has been realized? *Nat Med* 2005; **11**: 605.
9. Kawai T, Cosimi AB, Spitzer TR, *et al.* HLA-mismatched renal transplantation without maintenance immunosuppression. *N Engl J Med* 2008; **358**: 353.
10. Fudaba Y, Spitzer TR, Shaffer J, *et al.* Myeloma responses and tolerance following combined kidney and non-myeloablative marrow transplantation: in vivo and in vitro analyses. *Am J Transplant* 2006; **6**: 2121.
11. Scandling JD, Busque S, Dejbakhsh-Jones S, *et al.* Tolerance and chimerism after renal and hematopoietic-cell transplantation. *N Engl J Med* 2008; **358**: 362.
12. Pirenne J, Takahashi K, Kawai M, *et al.* Translation into the clinic of an experimentally proven pre-regulatory immunomodulatory protocol for intestinal transplantation: long-term results [abstract #O060]. *Transpl Int* 2007; **20**(S2): 17.
13. 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.
14. 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.
15. 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.
16. 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.
17. Kabelitz D, Geissler EK, Soria B, Schroeder IS, Fändrich F, Chatenoud L. Toward cell-based therapy of type I diabetes. *Trends Immunol* 2008; **29**: 68.
18. Renard C, Kristensen B, Gautschi C, Hruban V, Fredholm M, Vaiman M. Joint report of the first international comparison test on swine lymphocyte alloantigens (SLA). *Anim Genet* 1988; **19**: 63.
19. Strüber M, Hohlfeld JM, Kofidis T, *et al.* Surfactant function in lung transplantation after 24 hours of ischemia: Advantage of retrograde flush perfusion for preservation. *J Thorac Cardiovasc Surg* 2002; **123**: 98.
20. Yousem SA, Berry GJ, Cagle PT, *et al.* Revision of the 1990 working formulation for the classification of pulmonary allograft rejection: Lung Rejection Study Group. *J Heart Lung Transplant* 1996; **15**: 1.
21. Kamath AV, Morris ME. Functional expression of P-glycoprotein in the hepatic canalicular membrane of developing rats. *J Pharm Sci* 1998; **87**: 300.
22. Asperen JV, Tellingen OV, Beijnen JH. The pharmacological role of P-glycoprotein in the intestinal epithelium. *Pharmacol Res* 1998; **37**: 429.
23. Wacher VJ, Wu CY, Benet LZ. Overlapping substrate specifities and tissue distribution of cytochrome P450 3A and P-glycoprotein: Implications for drug delivery and activity in cancer chemotherapy. *Mol Carcinog* 1995; **13**: 129.
24. Schuetz EG, Beck WT, Schuetz JD. Modulators and substrates of P-glycoprotein and cytochrome P450 3A coordinately up-regulate proteins in human colon carcinoma cells. *Mol Pharmacol* 1996; **49**: 311.
25. Utsugi R, Barth RN, Lee RS, *et al.* Induction of transplantation tolerance with a short course of tacrolimus (FK506): I. Rapid and stable tolerance to two-haplotype fully MHC-mismatched kidney allografts in miniature swine. *Transplantation* 2001; **71**: 1368.
26. Warnecke G, Avsar M, Morancho M, *et al.* Preoperative low-dose irradiation promotes long-term allograft acceptance and induces regulatory T cells in a model of pulmonary transplantation. *Transplantation* 2006; **82**: 93.
27. Warnecke G, Avsar M, Steinkamp T, *et al.* Tacrolimus versus cyclosporine induction therapy in pulmonary transplantation in miniature swine. *Eur J Cardiothorac Surg* 2005; **28**: 454.
28. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 2005; **5**: 953.
29. Mantovani A, Sica A, Locati M. Macrophage polarization comes of age. *Immunity* 2005; **23**: 344.
30. 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.
31. 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.

