ORIGINAL ARTICLE

Thalidomide treatment prevents chronic graft rejection after aortic transplantation in rats – an experimental study

Katharine K. Miller[1,2,3,4], Dong Wang[1,2,3,4], Xiaomeng Hu[1,2,3,4], Xiaoqin Hua[1,3,4], Tobias Deuse[1,2,3,4,5], Evgenios Neofytou[6,7], Thomas Renne[8,9], Joachim Velden[10], Hermann Reichenspurner[3,4,5], Sonja Schrepfer[1,2,3,4,5,*] & Daniel Bernstein[11,*]

1 Transplant and Stem Cell Immunobiology (TSI)-Lab, University Heart Center Hamburg, Hamburg, Germany
2 Department of Surgery, Transplant and Stem Cell Immunobiology (TSI)-Lab, University California San Francisco (UCSF), San Francisco, CA, USA
3 Cardiovascular Research Center (CVRC), University Medical Center Hamburg-Eppendorf, Hamburg, Germany
4 German Centre for Cardiovascular Research (DZHK) e.V., University Medical Center Hamburg-Eppendorf, Hamburg, Germany
5 Department of Cardiovascular Surgery, University Heart Center Hamburg, Hamburg, Germany
6 Stanford Cardiovascular Institute, Stanford University School of Medicine, Stanford, CA, USA
7 Division of Cardiology, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA
8 Department of Clinical Chemistry, University Medical Center Hamburg, Hamburg, Germany
9 Department of Molecular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden
10 Evotec AG, Hamburg, Germany
11 Department of Pediatrics (Cardiology) and the Cardiovascular Institute, Stanford University, Stanford, CA, USA

SUMMARY

Cardiac allograft vasculopathy (CAV) affects approximately 30% of cardiac transplant patients at 5 years post-transplantation. To date, there are few CAV treatment or prevention options, none of which are highly effective. The aim of the study was to investigate the effect of thalidomide on the development of CAV. The effect of thalidomide treatment on chronic rejection was assessed in rat orthotopic aortic transplants in allogeneic F344 or syngeneic Lew rats (n = 6 per group). Animals were left untreated or received thalidomide for 30 days post-transplant, and evidence of graft CAV was determined by histology (trichrome and immunohistochemistry) and intragraft cytokine measurements. Animals that received thalidomide treatment post-transplant showed markedly reduced luminal obliteration, with concomitant rescue of smooth muscle cells (SMCs) in the aortic media of grafts. Thalidomide counteracted neointimal hyperplasia by preventing dedifferentiation of vascular SMCs. Measurement of intragraft cytokine levels after thalidomide treatment revealed downregulation of matrix metalloproteinase 8 and monocyte chemotactic protein 1, cytokines involved in tissue remodelling and inflammation, respectively. Importantly, no negative side effects of thalidomide were observed. Thalidomide treatment prevents CAV development in a rodent model and is therefore potentially useful in clinical applications to prevent post-transplant heart rejection.

Transplant International 2017; 30: 1181–1189

Key words
cardiac allograft vasculopathy, rats, thalidomide

Received: 24 April 2017; Revision requested: 23 May 2017; Accepted: 26 June 2017; Published online: 14 August 2017

doi:10.1111/tri.13004

Correspondence
Prof. Dr. med. Sonja Schrepfer, MD, PhD, Transplant and Stem Cell Immunobiology Lab (TSI), University Heart Center Hamburg, Campus Research (N27), Martinistr. 52, 20246 Hamburg, Germany
Tel.: +49 40 7410 59982;
e-mail: sonja.schrepfer@ucsf.edu

*Shared last authorship.

Introduction

Over the past several decades, patient survival after heart transplantation has improved; however, most of this benefit has accrued during the early period post-transplantation while long-term survival is still compromised by complications such as chronic cardiac allograft rejection, otherwise known as cardiac allograft vasculopathy (CAV). This form of chronic rejection – occurring months to years post-transplant – affects more than 30% of patients at 5 years post-transplant [1–3]. CAV is a highly aggressive form of coronary artery disease [4] caused by a combination of immune and nonimmune responses that result in characteristic narrowing of donor coronary arteries, although the exact mechanisms remain unclear. Of the few treatment options available for CAV, most focus on prevention and none are particularly effective [5]. To this date, the only treatment option for patients with CAV associated with longer lifespan is re-transplantation, which carries a higher risk compared to the original transplant [6].

Thalidomide was originally introduced as a therapy for morning sickness in pregnant women, but was soon removed from the market due to its teratogenic effects [7]. However, thalidomide has since been repurposed as an immunomodulator and has been approved for use as a treatment for multiple diseases, including multiple myeloma and erythema nodosum leprosum [8–10]. Thalidomide's anti-inflammatory and immunomodulatory functions as well as its beneficial effect on chronic graft-versus-host disease after bone marrow transplantation make it an ideal candidate for preventing graft rejection [11]. Indeed, thalidomide alone or low levels of thalidomide and cyclosporine can be used to reduce rejection of cardiac allografts in rabbits [12], and thalidomide has been found to lessen neotintimal thickness after aortic graft transplantation [13]. However, the mechanism by which thalidomide reduces rejection rates remains unknown, with reports conflicting regarding its effects on inflammatory cells, vascular pericytes and SMCs.

Here, we show effects of thalidomide treatment in a chronic rat orthotopic (Male Fischer 344:Male Lewis) aortic transplantation model. Thalidomide dramatically reduces the development of intimal thickening by rescuing SMC numbers, differentiation and localization. Intragraft cytokine levels were measured to further determine thalidomide's possible mechanisms of action.

Materials and methods

Animals

All animals were purchased from Charles River Laboratories (Bad Sulzfeld, Germany) and received care in compliance with the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996). Studies were approved by the local ethical committee 'Hamburg Amt für Gesundheit und Verbraucherschutz'.

Orthotopic aortic transplantation (chronic CAV study)

Orthotopic aortic transplantation was performed as previously described [14]. Briefly, a section of the thoracic aorta from F344 (allogeneic) or Lew (syngeneic) was orthotopically transplanted into the infra-renal abdominal aorta of Lew rats via an end-to-end anastomosis. Three groups (n = 6 animals per group) were randomly assigned to receive either 100 mg/kg/day of thalidomide p.o. or vehicle control.

Transplant International 2017; 30: 1181–1189

Heterotopic heart transplantation (acute study)

Heterotopic heart transplantation was performed as previously described [15]. Briefly, BN (allogeneic) and Lew (syngeneic) hearts were heterotopically transplanted to the abdominal great vessels of Lew rats. Three groups ($n = 6$ animals per group) were randomly assigned to receive either 100 mg/kg/day of thalidomide p.o. or vehicle control. Six days post-transplant, animals were euthanized, the apical half of the graft was snap frozen for cytokine quantification, and the caudal part was fixed (4% paraformaldehyde) for further tissue processing.

Histopathology

Harvested grafts were fixed (4% paraformaldehyde), dehydrated and embedded in paraffin. Each block was sectioned into 5-μm sections followed by trichrome staining using the manufacturer's protocol (Sigma-Aldrich, St. Louis, MO, USA) for evaluating luminal occlusion and immunofluorescence. Luminal obliteration was quantified, using IMAGEJ (Bethesda, MD, USA) as follows: Vascular occlusion (%) = [Area of intima/(Area of intima + Vascular lumen)] × 100. Three sections were analysed from each aortic allograft, and results were averaged.

Immunofluorescence staining

Paraffin sections underwent heat-induced antigen retrieval with Dako antigen retrieval solution (Dako, Glostrup, Denmark) followed by blocking with Image-iT FX signal enhancer (Invitrogen, Carlsbad, CA, USA). Primary antibodies were used as appropriate: smooth muscle actin (SMA) (ab5694), smooth muscle heavy chain (SMHC) (ab124679) or embryonic smooth muscle heavy chain (SMemb) (Yamasa 7602). A mouse irrelevant IgG1 (Abcam, Cambridge, UK) was used as negative control. Secondary antibodies were Alexa Fluor 488 or Alexa Fluor 555 (Invitrogen). Imaging was performed using a Nikon Eclipse TiE microscope equipped with the Perkin Elmer UltraVIEW VoX confocal imaging system. Analysis was carried out with VOLOCITY 6.1.1 (Perkin Elmer, Waltham, MA, USA).

Side effect screening

Serum was obtained prior to animal sacrifice in order to investigate the effect of thalidomide on blood cholesterol, triglycerides, kidney, liver and blood count.

Cytokine antibody array

Cytokine antibody arrays of homogenized grafts were performed according to the manufacturer's protocol (RayBiotech, Norcross, GA, USA). Membranes were digitized using bioluminescence imaging and quantified using IMAGEJ. Cytokine concentrations are expressed in arbitrary units (AU).

Unidirectional ELISpot assay

Recipient splenocytes (responder cells) were isolated from fresh spleen 6 days after heart transplantation. 1×10^6 donor stimulator cells were incubated with 1×10^6 responder cells for 24 h. IFNγ spot frequencies were assessed in quadruplicate using an automatic counted ELISpot plate reader (CTL, Cincinnati, OH, USA).

CFSE-MLR proliferation assay

The carboxyfluorescein succinimidyl ester-mixed lymphocyte reaction (CFSE-MLR) was performed with the acute heterotopic transplantation study animals. Briefly, responder and stimulator cells were co-cultured at equal ratios (3×10^6/ml) in a 48-well plate for 5 days at 37 °C, 5% CO_2 in RPMI 1640 medium (Gibco, Darmstadt, Germany) supplemented with 1% penicillin/streptomycin solution, 10% FCS and 50 μM β-mercaptoethanol (ES-007-E; Millipore, Darmstadt, Germany). Cells were harvested and analysed by flow cytometry, gating for intensity of CFSE fluorescence. Stimulation index (SI) was calculated based on the mean value of each animal.

Statistics

Data are presented as mean ± standard deviation. Comparisons within groups used analysis of variance with Bonferroni or LSD *post hoc* tests, as appropriate. Probability values (P) less than 0.05 were considered significant. Statistical analysis was performed using SPSS statistical software package 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Thalidomide treatment reduces luminal obliteration in chronic rejection model

We measured the effect of thalidomide on intimal thickening in a chronic low responder model using an accepted orthotopic aortic transplantation model for

CAV [14,16]. Syngeneic transplants were performed as a procedural control. Allogeneic transplants were given either vehicle control or 100 mg/kg thalidomide by oral gavage for 30 days post-transplant (Fig. 1a).

As expected, syngeneic grafts showed no signs of cellular rejection in histology and exhibited minimal levels of luminal obliteration (1.32%) as compared to vehicle-treated allogeneic grafts (21.27%). Strikingly, animals treated with thalidomide showed marked reduction in luminal occlusion (6.76%, $P < 0.001$) (Fig. 1b/c), suggesting that thalidomide may be a viable option for preventing CAV.

SMCs of the aortic media are maintained by thalidomide in CAV model

Staining for smooth muscle actin (SMA) confirms that the aortic media of syngeneic grafts is largely comprised of mature SMCs (Fig. 2a). A strong reduction in medial SMA intensity was found in vehicle-treated allogeneic grafts, an effect that was prevented by thalidomide treatment. Quantification of immunohistochemical images confirmed these observations (Fig. 2c).

To clarify SMC differentiation levels in each model, we compared smooth muscle cell heavy chain (SMHC; marker for differentiated SMCs) and embryonic smooth muscle heavy chain (SMemb; marker for dedifferentiated SMCs) intensities (Fig. 2b/c). Our images visually and quantifiably revealed that syngeneic transplants have organized differentiated medial SMCs and low levels of dedifferentiated intimal SMCs. In contrast, vehicle-treated allogeneic transplants had decreased differentiated SMC levels and increased dedifferentiated SMCs. Thalidomide treatment strongly attenuated these effects, reducing dedifferentiated intimal cells to control

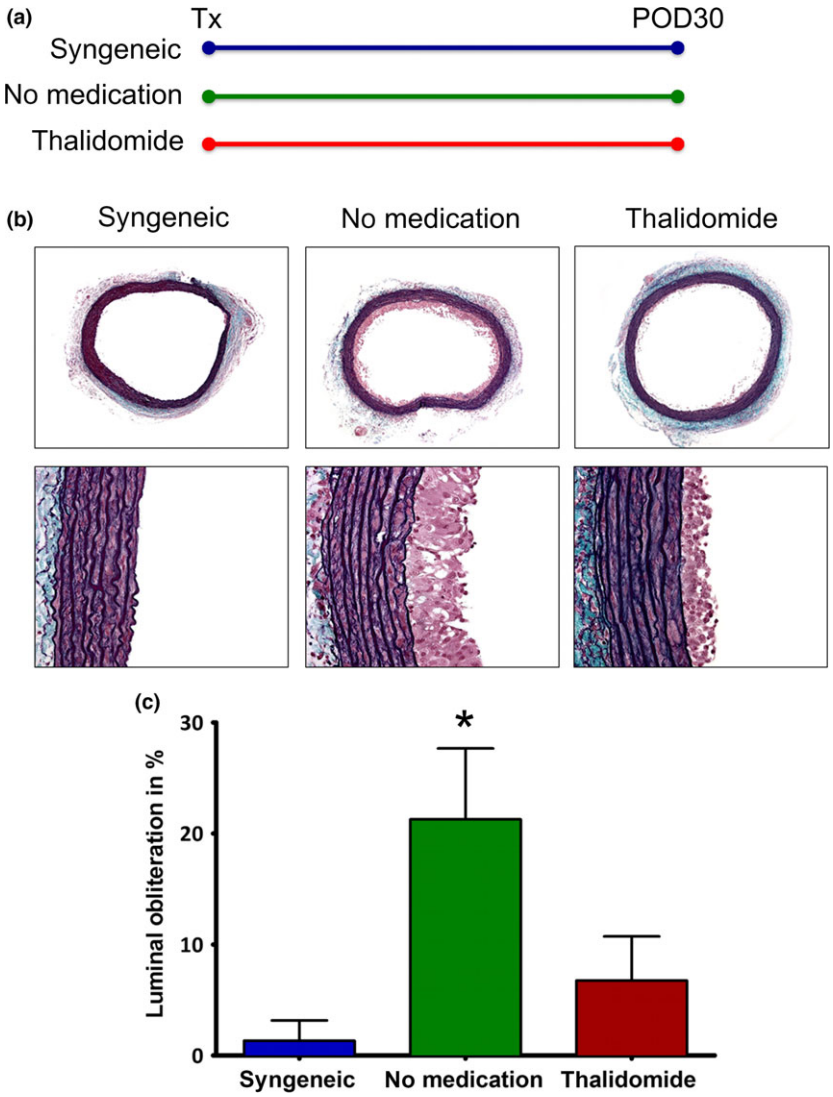

Figure 1 Treatment with thalidomide reduces luminal obliteration. (a) Overview of experimental protocol. Rats underwent either syngeneic (blue) or allogeneic (green and red) orthotopic aortic transplants. Allogeneic transplant recipients received either no medication (vehicle control, green) or thalidomide (100 mg/kg; red) by oral gavage for 30 days post-transplant. (b) Representative Masson's trichrome-stained images of aortic luminae after treatment regime show that the increased luminal obliteration after allogeneic transplant is drastically reduced by thalidomide treatment (top row: 50× magnification; bottom row: 400× magnification). (c) Quantification of luminal obliteration confirms levels of hyperplasia. The syngeneic model shows low base levels of obliteration (mean = 1.34%), which is strongly increased in the allogeneic no medication control (mean = 21.27%) and strikingly mitigated upon treatment with thalidomide (mean = 6.76%; $P < 0.001$). Error bars indicate mean ± SD. *$P < 0.05$.

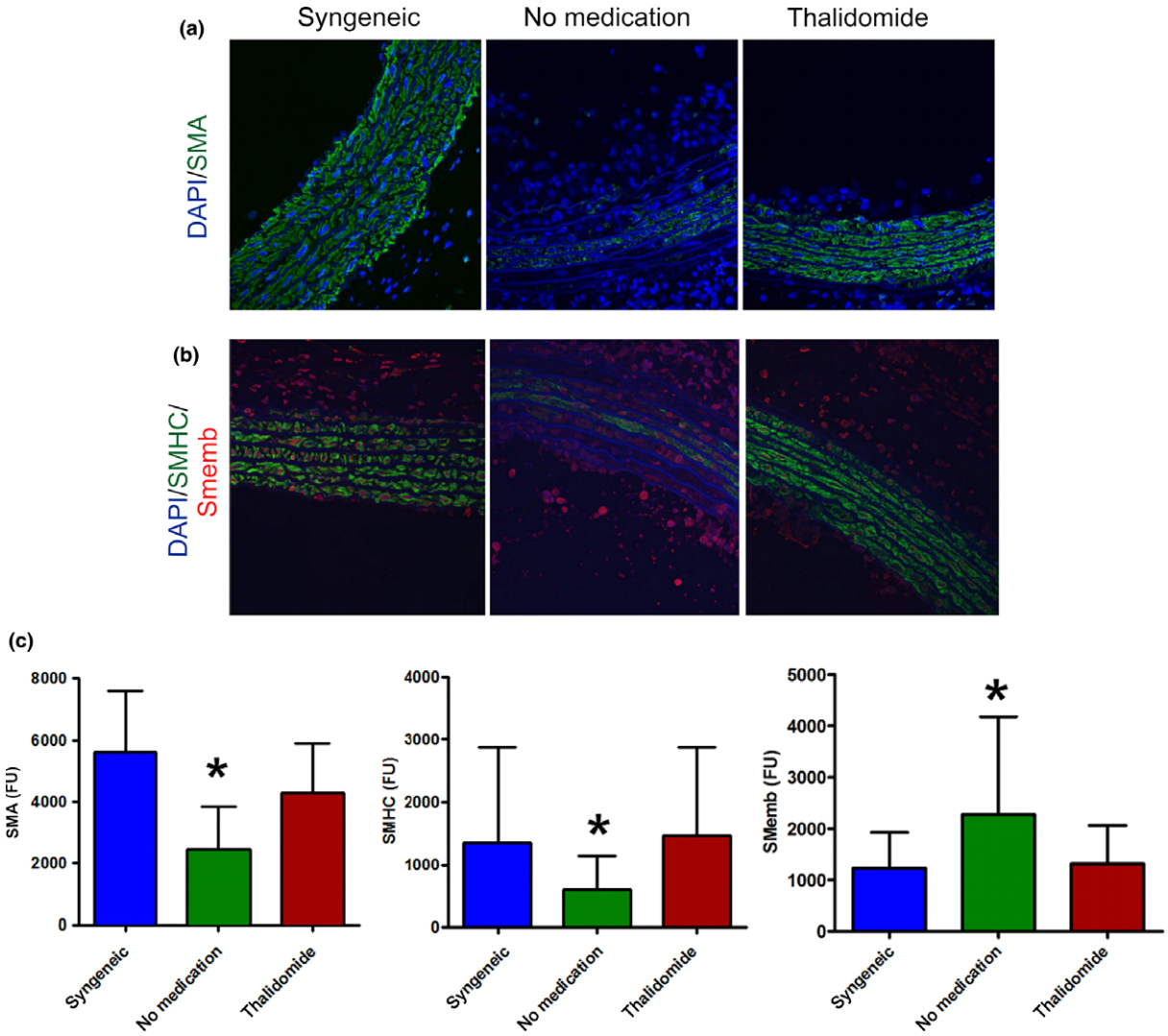

Figure 2 SMC number, differentiation and localization are maintained by thalidomide treatment. (a) Mature SMCs stained with SMA (green) are shown organized at the aortic media in syngeneic model. While allogeneic no medication control-treated animals show a disorganized media with a reduction in the number of SMCs, treatment with thalidomide shows a clear retention of both number and localization of SMCs (400× magnification). (b) Allogeneic transplant shows an increase in dedifferentiated SMCs (SMemb; red) which correlates with a parallel loss of differentiated SMCs (SMHC; green). Thalidomide treatment maintains differentiation and localization of SMCs (400× magnification). (c) Quantification of fluorescence further confirms the retention of mature SMC by thalidomide treatment (SMA, SMHC) and dedifferentiated SMC (SMemb) levels. Error bars indicate mean ± SD. $*P < 0.05$.

levels with a corresponding increase in differentiated medial SMCs.

Thalidomide treatment alters intragraft cytokine release profile

To investigate the mechanism by which thalidomide affects the proliferative and pathological features of CAV, we examined expression of key cytokines/chemokines induced in allogeneic graft transplantation (Fig. 3). Cytokine protein arrays showed that several cytokines, including MMP-8, TIMP-1, MCP-1 and ICAM-1, were significantly upregulated in the vehicle-treated allogeneic group versus controls. Thalidomide reduced expression levels of MMP-8 ($P < 0.05$) and MCP-1 ($P < 0.01$), cytokines associated with tissue remodelling and inflammation, respectively. Reduction of these cytokines may be indicative of thalidomide's mechanism of action: decreased tissue remodelling, as seen in our histological results, as well as reduced post-transplant inflammation.

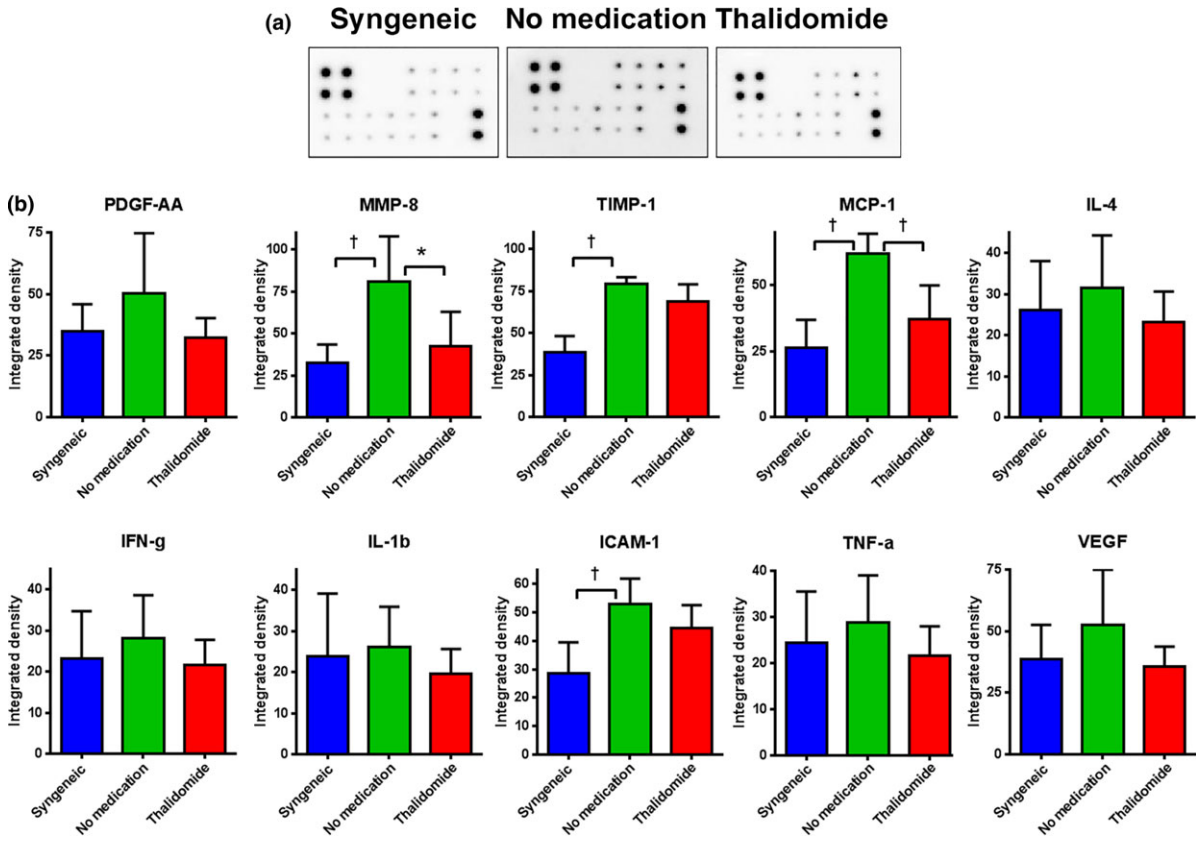

Figure 3 Cytokine profiles of grafts show thalidomide treatment reduces levels of specific cytokines. (a) Representative images of intragraft cytokine profiles for each graft type. (b) Quantification of cytokine profiles reveals that expression levels of MMP-8, TIMP-1, MCP-1 and ICAM-1 are upregulated after allogeneic transplant with no medication. MMP-8 and MCP-1 expression are reduced in the thalidomide treatment model. Error bars indicate mean ± SD. $^{*}P < 0.05$. $^{\dagger}P < 0.01$.

No significant side effects caused by thalidomide treatment in rats

We tested the safety of thalidomide after allogeneic orthotopic aortic transplants. Animals treated with thalidomide showed no obvious signs of discomfort or distress. Blood samples from vehicle-treated ($n = 3$) or thalidomide-treated ($n = 7$) animals showed no significant differences in biomarkers of kidney function (BUN, creatinine), serum cholesterol and triglyceride levels (cholesterol, triglyceride, LDL, HDL), liver function (ALT, AST) or whole blood count (RDW, Leucocyte, PLT, Hb, Hct, RBC, MCV, MCH, MCHC) (Fig. S1).

Thalidomide treatment does not attenuate lymphocyte activity in acute transplant rejection model

As our data support thalidomide as a potential therapy for CAV, we aimed to clarify whether the success of thalidomide treatment was due to a general reduction in graft rejection or whether it was specific to chronic rejection. Thus, we tested the effect of thalidomide treatment in heterotopic heart transplantation, an accepted rodent model for acute transplant rejection [15]. Syngeneic (Lew:Lew) or allogeneic (BN:Lew) transplantation was performed (Fig. S2a). Allogeneic transplant recipients received either vehicle control or thalidomide post-transplant. At 6 days postoperation (POD6), splenocytes were collected for ELISpot and mixed lymphocyte reaction (MLR) assays.

IFNγ levels – a proxy for systemic lymphocyte activation – were measured by ELISpot. In contrast to the dramatic effects of thalidomide in our chronic rejection model, thalidomide treatment did not appear to affect IFNγ production in our acute rejection model (Fig. S2b). Similarly, no alterations in lymphocyte proliferation were found by our MLR assay (Fig. S2c). These data suggest that thalidomide may be a specific treatment for chronic rejection, as it does not appear to be beneficial for reduction of acute rejection.

The primary pathology in CAV is coronary vessel intimal thickening; thus, reducing luminal obliteration

would be highly advantageous to the long-term survival of transplant recipients.

Discussion

Cardiac allograft vasculopathy is a leading cause of late death for cardiac transplant recipients and has remained resistant to therapeutic interventions. The identification of thalidomide as an immunomodulatory agent suggests its potential use in preventing graft rejection. Thalidomide has been described as having anticytokine, antiintegrin and antiangiogenic properties [17], and has beneficial effects in other proliferative diseases, including as a treatment for idiopathic pulmonary fibrosis, bone marrow transplantation, skin allograft transplantation and heterotopic heart transplantation [12,18,19]. As thalidomide's complete mechanism of action is yet unclear, we have investigated the pathophysiological effects of thalidomide in a rat aortic model of CAV. In this model, analysis of histopathological specimens is drastically facilitated as transplant vasculopathy may be examined in one single vessel of a sole defined diameter instead of the exploration of numerous small cardiac vessels showing a vast variety in size in the heterotopic heart transplant model. Indeed, the immune response generated by an aortic allograft is sufficient to trigger chronic alterations in the transplant [20].

Our data suggest that thalidomide dramatically reduces luminal narrowing in an established rat model of CAV. Immunohistochemical analysis showed that loss of medial differentiated SMCs – a hallmark of CAV – was prevented by thalidomide treatment. Furthermore, thalidomide prevented dedifferentiation and intimal proliferation of SMCs. Our results are supported by recent literature showing beneficial effects of thalidomide in rat models of transplant arteriosclerosis and hereditary haemorrhagic telangiectasia [13,21].

Because the molecular mechanism of thalidomide is not yet clear, we generated an intragraft cytokine profile, showing that thalidomide significantly reduced expression of MMP-8 and MCP-1. Zhang *et al.* [13] used immunohistochemistry and Western blot to suggest that thalidomide alters VEGF, PDGF and ICAM-1 levels, whereas we found no significant alteration in these molecules using the more accurate ELISpot and MLR assays. This difference in observation is likely due to the fact that Zhang *et al.* used a high responder transplantation model (BN:Lew) in their assays, which does not accurately represent CAV, while we used a more appropriate low responder model (F344:Lew) to simulate chronic rejection.

Matrix metalloproteases (MMPs) are zinc enzymes involved in extracellular matrix turnover. Interestingly, increased MMP expression levels have been connected with intimal thickening [22] and increased levels of MMP-2 and MMP-9 have been associated with chronic graft rejection [22]. MMP-8 itself appears to largely be produced by neutrophils and is upregulated within the first few weeks post-transplantation [23]. Furthermore, general inhibition of MMPs prevents migration and proliferation of SMCs in CAV [24]. An MMP inhibitor that has been shown to reduce levels of MMP-8 was also linked to attenuation of CAV [24]. This is consistent with our findings, which show downregulation of MMP-8 associated with thalidomide treatment and reduced SMC proliferation and dedifferentiation.

Intragraft cytokine MCP-1 levels were also significantly reduced by thalidomide treatment. The literature surrounding MCP-1's function supports this data. Monocyte chemotactic protein-1 (MCP-1) is associated with inflammatory responses, namely recruiting monocytes, memory T cells and dendritic cells to places of inflammation. Reduced levels of MCP-1 have been correlated with reduction of neointimal hyperplasia [25,26] and decreased chronic cardiac rejection [27].

Because of thalidomide's previously identified teratogenic effects [7], it was vital that we examine the potential side effects of thalidomide treatment. As seen with previous approval of thalidomide for treatment of erythema nodosum leprosum, we found no significant side effects to our thalidomide treatment in rats. However, the study period was only 28 days, thus lacking long-term data for safety and toxicity of thalidomide.

Finally, to further elucidate thalidomide's function, we wanted to clarify whether the effect we saw was specific to CAV or due to general immunogenic suppression. We examined whether thalidomide affected the pathogenic pathways associated with acute transplant rejection, namely T lymphocyte activation and proliferation. In contrast to the dramatic effects seen on CAV pathophysiology, we did not see significant inhibition of lymphocyte activation or proliferation. This leads us to conclude that thalidomide likely does not reduce CAV via prevention of generalized immune activation, but rather through a different, more specific mechanism of action on the vascular wall.

In conclusion, we demonstrate that thalidomide treatment dramatically reduces CAV in a well-established rat model of chronic rejection, preventing the intimal

proliferation and the loss of medial differentiated SMCs that are hallmarks of CAV. Thalidomide is therefore potentially useful in clinical applications to prevent CAV after human heart transplantation.

Authorship

DB and SS: concept was incepted. SS: project development and experiments were overseen. KKM and EN: manuscript preparation. KKM: additional scientific input and analysis were given. DW, XH, XH, TD, TR and JV: experiments were performed. HR: further scientific guidance was given. DB and SS: final manuscript review was performed.

Funding

This study was funded by the German Research Foundation (Deutsche Forschungsgemeinschaft; DFG: SCHR992/3-1 and SCHR992/4-1; S.S. and DE2133/2-1; T.D.), the International Society for Heart and Lung Transplantation (ISHLT; K.K.M., D.W. and S.S.), the Else Kröner Excellence Stipend from the Else-Kröner-Fresenius-Stiftung (2012_EKES.04) and the National Institutes of Health (NIH) grant R21 HL123655 (D.B.). D.W. was supported by the Max Kade Foundation.

Conflict of Interest

The authors have declared no conflicts of interest.

Acknowledgements

We thank Christiane Pahrmann for her technical assistance.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Thalidomide treatment did not cause significant side effects.

Figure S2 Thalidomide treatment does not mitigate lymphocyte activity in acute rejection model.

REFERENCES

1. Taylor DO, Edwards LB, Boucek MM, *et al.* Registry of the International Society for Heart and Lung Transplantation: twenty-fourth official adult heart transplant report–2007. *J Heart Lung Transplant* 2007; **26**: 769.
2. Colvin-Adams M, Agnihotri A. Cardiac allograft vasculopathy: current knowledge and future direction. *Clin Transplant* 2011; **25**: 175.
3. Lindenfeld J, Miller GG, Shakar SF, *et al.* Drug therapy in the heart transplant recipient: part I: cardiac rejection and immunosuppressive drugs. *Circulation* 2004; **110**: 3734.
4. Costello JP, Mohanakumar T, Nath DS. Mechanisms of chronic cardiac allograft rejection. *Tex Heart Inst J* 2013; **40**: 395.
5. Schmauss D, Weis M. Cardiac allograft vasculopathy: recent developments. *Circulation* 2008; **117**: 2131.
6. Johnson MR, Aaronson KD, Canter CE, *et al.* Heart retransplantation. *Am J Transplant* 2007; **7**: 2075.
7. Franks ME, Macpherson GR, Figg WD. Thalidomide. *Lancet* 2004; **363**: 1802.
8. Singhal S, Mehta J, Desikan R, *et al.* Antitumor activity of thalidomide in refractory multiple myeloma. *N Engl J Med* 1999; **341**: 1565.
9. Sheskin J. Thalidomide in the treatment of lepra reactions. *Clin Pharmacol Ther* 1965; **6**: 303.
10. Matthews SJ, McCoy C. Thalidomide: a review of approved and investigational uses. *Clin Ther* 2003; **25**: 342.
11. Lim SH, McWhannell A, Vora AJ, Boughton BJ. Successful treatment with thalidomide of acute graft-versus-host disease after bone-marrow transplantation. *Lancet* 1988; **1**: 117.
12. Carvalho JB, Petroianu A, Travolo E, de Oliveira BH, Duarte AB, Alberti LR. Effects of immunosuppression induced by thalidomide and cyclosporine in heterotopic heart transplantation in rabbits. *Transplant Proc* 2007; **39**: 1640.
13. Zhang Y, Yang M, Yang Y, *et al.* Thalidomide attenuates graft arteriosclerosis of aortic transplant in a rat model. *Transplant Proc* 2011; **43**: 2022.
14. Stubbendorff M, Deuse T, Hammel A, Robbins RC, Reichenspurner H, Schrepfer S. Orthotopic aortic transplantation: a rat model to study the development of chronic vasculopathy. *J Vis Exp* 2010; Dec 4;(46). pii: 1989. doi: 10.3791/1989.
15. Deuse T, Hua X, Taylor V, *et al.* Significant reduction of acute cardiac allograft rejection by selective janus kinase-1/3 inhibition using R507 and R545. *Transplantation* 2012; **94**: 695.
16. Deuse T, Hoyt G, Koyanagi T, Robbins RC, Schrepfer S. Prevention and inhibition but not reversion of chronic allograft vasculopathy by FK778. *Transplantation* 2008; **85**: 870.
17. Peuckmann V, Fisch M, Bruera E. Potential novel uses of thalidomide: focus on palliative care. *Drugs* 2000; **60**: 273.
18. Ye Q, Chen B, Tong Z, *et al.* Thalidomide reduces IL-18, IL-8 and TNF-alpha release from alveolar macrophages in interstitial lung disease. *Eur Respir J* 2006; **28**: 824.
19. Chaves DN, Petroianu A, Alberti LR, Pereira WA. Effects of thalidomide, cyclosporine, and diclofenac on skin allograft survival in rabbits. *Transplant Proc* 2004; **36**: 1018.
20. Mennander A, Tiisala S, Halttunen J, Yilmaz S, Paavonen T, Hayry P. Chronic rejection in rat aortic allografts. An experimental model for transplant arteriosclerosis. *Arterioscler Thromb* 1991; **11**: 671.
21. Lebrin F, Srun S, Raymond K, *et al.* Thalidomide stimulates vessel maturation and reduces epistaxis in individuals with hereditary hemorrhagic telangiectasia. *Nat Med* 2010; **16**: 420.

22. Suzuki J, Isobe M, Kawauchi M, Endoh M, Amano J, Takamoto S. Altered expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in acutely rejected myocardium and coronary arteriosclerosis in cardiac allografts of nonhuman primates. *Transpl Int* 2000; **13**: 106.
23. Aharinejad S, Krenn K, Zuckermann A, *et al.* Serum matrix metalloprotease-1 and vascular endothelial growth factor – a predict cardiac allograft rejection. *Am J Transplant* 2009; **9**: 149.
24. Hariya A, Takazawa K, Yamamoto T, Amano A. ONO-4817, a novel matrix metalloproteinase inhibitor, attenuates allograft vasculopathy in a rat cardiac transplant. *J Heart Lung Transplant* 2004; **23**: 1163.
25. Ge S, Zhou G, Cheng S, *et al.* Anti-atherogenic effects of montelukast associated with reduced MCP-1 expression in a rabbit carotid balloon injury model. *Atherosclerosis* 2009; **205**: 74.
26. Ishihara T, Haraguchi G, Konishi M, *et al.* Effect of adiponectin on cardiac allograft vasculopathy. *Circ J* 2011; **75**: 2005.
27. Itoh S, Nakae S, Axtell RC, *et al.* IL-17 contributes to the development of chronic rejection in a murine heart transplant model. *J Clin Immunol* 2010; **30**: 235.