TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

LETTER TO THE EDITORS

Mesenchymal stromal cells promote bowel regeneration after intestinal transplantation: myth to mucosa

doi:10.1111/tri.12139

Dear Sirs,

We report the case of a 52-year-old female patient who underwent an isolated small bowel transplant and subsequently received mesenchymal stromal cells (MSCs) to treat severe, refractory bowel dysfunction secondary to infection. The patient has a background of enteric neuromyopathy and short gut syndrome and was on home parenteral nutrition for 22 years. She received an isolated intestinal graft from a donor after brain death with a human leucocyte antigen mismatch of 0–0–0. Induction immunosuppression was with 30 mg of intravenous Alemtuzumab (Campath) with the same dose repeated 24 h later. Maintenance immunosuppression was achieved with Tacrolimus (Prograf) aiming for trough levels of 8–12 ng/ml.

Immediate graft function with independence from total parenteral nutrition (TPN) was achieved by day 24 post-transplant and this continued up to the 10th month after transplantation. The postoperative course was otherwise complicated by acute kidney injury secondary to calcineurin inhibitor toxicity and post-transplant lymphoproliferative disease (PTLD). PTLD manifest at 60 days post-transplant, when a routine surveillance biopsy of the transplant graft demonstrated dense nodular lymphoid tissue and Epstein–Barr virus (EBV)-positive cells. A positron emission tomography (PET) CT scan confirmed the presence of inflamed mesenteric lymph nodes. This was successfully treated with four doses of 375 mg/m^2 of Rituximab per dose. No other immunosuppressive strategy was applied. Tacrolimus levels were kept the same since she was on monotherapy and we did not want to risk rejection in the bowel. The patient did not receive any antiviral therapy after transplantation as she was cytomegalovirus negative, as was the donor. Maintenance immunosuppression also proved to be troublesome. Tacrolimus was administered for the first five postoperative months and was converted to sirolimus owing to a serum creatinine of 580 mmol/l. This had to be discontinued because of severe oral and stomal ulceration and mycophenolate mofetil (MMF) and prednisolone were initiated. Basiliximab, 20 mg, was administered as an induction agent to facilitate the conversion of agents and was continued monthly for the subsequent 4 months.

At 10 months post-transplantation, the patient presented with increased stoma output to 40 ml/kg/24 h. Graft histology showed severe enteritis, extensive loss of surface epithelium with oedematous lamina propria and predominantly inflammatory infiltrate. Histology from the stoma effluent showed Candida and Norovirus infections. Forty-five days of bowel dysfunction ensued and despite prolonged treatment with the maximum dose of fluconazole and full supportive care, there was no endoscopic or histological evidence of regeneration. The patient was re-established on TPN and MMF was switched to azathioprine in an attempt to reduce immunosuppression. This was discontinued as histology demonstrated no evidence of regeneration in the villi. Tacrolimus was re-instituted, but no levels were achieved attributable to poor absorption. Despite the above measures, at 355 days after transplantation, the patient demonstrated graft dysfunction both clinically and macroscopically with no signs of improvement. Immunosuppressive options had been exhausted and with the patient re-established on TPN, line access was becoming problematic with recurrent line infections and thrombosed central veins. It was at this stage that MSC transplantation was considered for the dual properties of immunosuppression and tissue repair and was based on the evidence of their use in inflammatory bowel disease (IBD) [1,2]. MSCs were freshly harvested from the bone marrow of a third party allogeneic donor. One million cells/kg were infused via a central vein over 15 min. The infusion was well tolerated with no immediate or late side effects. At 4 days following MSC infusion, the patient's GI output decreased to 25 ml/kg/24 h and her serum citrulline increased to 15.4 μmol/l (having previously ranged between 5 and 9 μmol/l). An endoscopy performed 11 days following administration of MSCs demonstrated a marked improvement macroscopically, with rudimentary villi. The histological findings demonstrated marked focal regenerative changes as shown in Fig. 1. Stoma output continued to

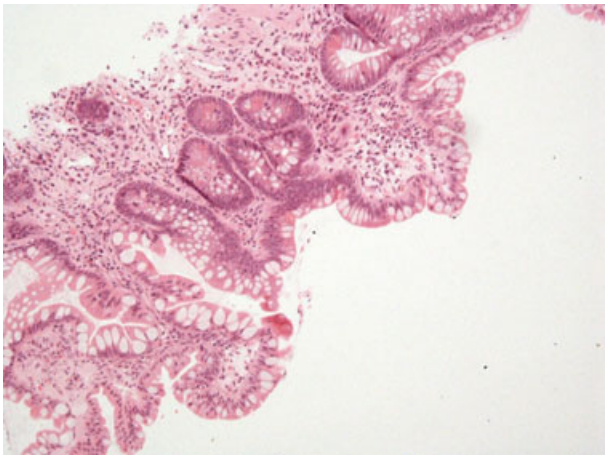

Figure 1 Bowel histology 11 days after mesenchymal stromal cell administration showing marked focal regenerative changes with return of villi although they are sparse and stunted. The sparse areas appear to be interspersed non villous, flat regenerative epithelium. The previous granulation tissue-like appearances of the lamina propria have become near normal connective tissue. The crypts also demonstrate regenerative multiple budding with increased mitosis, but no significant apoptosis.

decrease (15–20 ml/kg/24 h) and become thicker in consistency, with increasing enteral intake. TPN was weaned off in the next few days and nutritional parameters including serum magnesium and albumin levels showed signs of improvement. At 60 days following administration, the patient continued to have reduced GI output and was clinically well. Her serum citrulline level was 30.2 μmol/l. Tacrolimus was re-instituted with evidence of absorption and levels targeted between 5 and 7 ng/ml. Her serum creatinine ranged between 100 and 120 mmol/l. With regard to PTLD status after administration of MSCs, serum levels of EBV remained undetected on routine regular testing and a repeat PET CT scan performed at 75 days after administration showed no evidence of PTLD.

Mesenchymal stromal cells are heterogeneous cells which can be derived from the connective component of virtually any tissue [3]. They have the ability to differentiate many mesodermal lineages and this highlights their potential for use in regenerative medicine [4]. Although there is some evidence that their differentiation potential might be exploited to promote tissue repair in several conditions [5,6], the most promising feature of MSCs for clinical use is the ability to immunosuppress immune cells [7]. This does not require MSCs and the target immune cell to be histo-compatible thus enabling them to be transplanted across genetic barriers [8]. In the literature, MSCs have been administered intravenously, intra-enterally via the donor superior mesenteric artery (SMA), or intra-peritoneally [9]. With intravenous administration, MSCs may not reach the desired destination, with 'trapping' of cells in the lungs [10]. Transplanting the cells via the donor SMA appeared to be the most logical but the risk of embolism was a concern. The decision to transplant the MSCs intravenously was based on the fact that this route is the best evidenced and is reported to be well tolerated [9,10].

This is the first reported case of the use of MSCs in intestinal dysfunction after small bowel transplantation. Simplistically, we believe that whatever the cause of bowel dysfunction, the root cause is the inflammatory process at the mucosal level that hampers the turnover of the much required villous structures. We have demonstrated that MSC therapy in the setting of inflammation from Candida and Noroviral infection was effective in triggering a regenerative process. It was well tolerated and early results are promising. However, further studies need to be performed to ascertain the efficacy and reproducibility of this exciting new therapy in bowel transplant patients experiencing bowel dysfunction from infection or perhaps rejection.

Carlo D. L. Ceresa, Roger N. Ramcharan,
Peter J. Friend and Anil Vaidya
The Oxford Transplant Centre, Churchill Hospital, Oxford, UK
e-mail: carloceresa@hotmail.com

Conflicts of interest

The authors of this manuscript have no conflicts of interest to disclose.

Funding

There was no external funding for this project.

Acknowledgements

Thanks to Helene Mellor of the Illustration Services at the Cellular Pathology Department of the John Radcliffe Hospital, Oxford for providing us with the photographs of the histology slides.

References

1. Liang J, Zhang H, Wang D, *et al.* Allogeneic mesenchymal stem cell transplantation in seven patients with refractory inflammatory bowel disease. *Gut* 2012; **61**: 468.
2. Duijvestein M, Wildnberg ME, Vos ACW, *et al.* Phase I results of intravenous autologous bone marrow derived mesenchymal stromal cell treatment in refractory Crohn's disease. *J Crohn's Colitis* 2010; **4**: S6.
3. Trento C, Dazzi F. Mesenchymal stem cells and innate tolerance: biology and clinical applications. *Swiss Med Wkly* 2010; **140**: w13121.

4. Jiang Y, Jahagirdar BN, Reinhardt RL, *et al.* Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002; **418**: 41.
5. Wakitani S, Imoto K, Yamamoto T, *et al.* Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage* 2002; **10**: 199.
6. Chen SL, Fang WW, Ye F, *et al.* Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol* 2004; **94**: 92.
7. Larsen S, Lewis ID. Potential therapeutic applications of mesenchymal stromal cells. *Pathology* 2011; **43**: 592.
8. Le Blanc K, Tammik L, Sundberg B, *et al.* Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibilty complex. *Scand J Immunol* 2003; **57**: 11.
9. Garcia-Bosch O, Ricart E, Panes J. Review article: stem cell therapies for inflammatory bowel disease – efficacy and safety. *Aliment Pharmacol Ther* 2010; **32**: 939.
10. Lee RH, Pulin AA, Seo MJ, *et al.* Intravenous hMSCs improve myocardial infarction in mice because cells embolised in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell* 2009; **5**: 54.

