Our panel of experts highlight the most important research articles across the spectrum of topics relevant to the field of regenerative medicine

Therapeutic allogeneic MSCs are cleared by macrophages after administration

Evaluation of: Arutyunyan I, Elchaninov A, Fatkhudinov T *et al.* Elimination of allogeneic multipotent stromal cells by recipient macrophages in different models of regeneration. *Int. J. Clin. Exp. Pathol.* 8, 4469–4480 (2015).

Allogeneic multipotent mesenchymal stromal cells (MSCs) have long been considered immunoprivileged based on their lack of class II HLA expression, and because they are able to suppress T-cell proliferation *in vitro*. The dogma for therapeutic MSCs was that once injected, they would home to a site of injury undetected by the immune system, and engraft and differentiate site-specifically to regenerate the injured tissue. This dogma has been challenged recently by studies suggesting that allo-MSCs act through paracrine signaling rather than differentiation and engraftment, and although they have a therapeutic window, are rapidly eliminated by the recipient immune system. A critical question, then, is how allogeneic MSCs are eliminated after administration. Arutyunyan addressed this question by conducting translational animal studies that recapitulated liver failure and critical limb ischemia (CLI), two conditions that have been treated clinically with MSCs.

In one model, allogeneic MSCs were labeled with the fluorescent vital dye, PKH26, and were then injected either intrasplenically or intrahepatically after partial hepatectomy in rats. In the second model, labeled allogeneic MSCs were injected into the site of injury after induction of CLI in

the calf muscle of rats. Samples of splenic, hepatic and muscle tissue were harvested post-transplantation at various time points after MSC injection, and were analyzed for PKH26 fluorescence, and for the colocalization of PKH26 with the macrophage-specific marker, CD68. The PKH26 label colocalized almost completely with CD68 in the spleen after intrasplenic injection, suggesting massive phagocytosis of the allogeneic MSCs or their membranous debris by recipient macrophages. In the same intrasplenic injection model, the PKH26-labeled MSCs appeared to home to the regenerating liver and colocalized with CD68 there. Elimination was most potent in spleen, which is rich in activated macrophages. This result is important clinically, as delivery of MSCs through the spleen is generally considered to be a promising route of administration (ROA) of therapeutic MSCs to the liver, with which it shares a blood supply. Using another common ROA, direct intrahepatic injection, showed lower rates of elimination, although MSCs were also eliminated through phagocytosis by macrophage in that instance as well. In muscle, label was retained much longer than in spleen or liver, despite ongoing chronic inflammation. Although the authors are cautious about their interpretation, these data suggest that elimination of labeled MSCs occurs through phagocytosis by recipient macrophages, which is supported by a recent study that demonstrated complement activation by allogeneic MSCs, and subsequent opsonization, or ‘tagging’ of the cells for elimination by phagocytes [1]. Opsonization would most certainly lead to elimination of live and lysed allo-MSCs by macrophages, but not before, as another recent study sug-

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gests, the contents of the intact and lysed MSCs deliver their therapeutic effect [2].

GLT1 overexpressing astrocytes role in contusion transplant models

Evaluation of: Li K, Javed E, Scura TJ, Seetharam S, Falnikar A, Richard JP, Chorath A, Maragakis NJ, Wright MC, Lepore AC. Human iPS cell derived astrocyte transplants preserve respiratory function after spinal cord injury. *Exp. Neurol.* 27, 479–492 (2015).

Neural stem cell transplantation after spinal cord injury is not a new idea. Secondary damage after spinal cord injury is also an event that must be controlled. Injury produces a loss of tissue due to primary damage and also due to glutamate excitotoxicity which may be caused by the lack of glial influences that have also been damaged as a result of the trauma. Researchers in the current study in *Experimental Neurology* decided to interrogate the role of astrocytes in secondary injury by transplanting human induced pluripotent stem cells (iPSCs) that have been driven to an astrocytic fate (hIPSA). The researchers importantly characterized the hIPSA and transduced them to express functional GLT1, a glutamate transporter, using lentiviral technology. Transplantation of the hIPSA into a spinal cord contusion models indicated the cells survived out to week four post implant and co-labeled with astrocytic and human antigen markers. Control cells also survived four weeks post injury as well. All cells overexpressing lentiviral GLT1 continued to express GLT1 protein after transplantation four weeks post implant. Importantly, the transplants did not have uncontrollable proliferation or form tumors in the recipient animals. These experiments were performed in rats and mice with similar results which will be beneficial in future studies involving transgenic mice.

Li and colleagues assessed the therapeutic efficacy of hIPSA implantation in the contusion model. GLT1 expressing iPSCs had a reduction in lesion size and volume compared with appropriate controls. The protective effect was in the epicenter where most of the damage occurs with contusion. The neuromuscular junction (NMJ) was also preserved with GLT1 overexpressing hIPSA transplants which are crucial to diaphragm connectivity and breathing. Extensive quantitation determined GLT1-hIPSA transplant recipients had a reduction in the incidence of partial innervations compared with control groups. Functional EMG activity was accessed and all animals demonstrated a reduction compared with uninjured animals. Amplitude of EMG bursts in the GLT1 expressing hIPSA recipients was increased. Phrenic nerve activity measured by

compound muscle action potentials was also increased in GLT1-overexpressing hIPSA recipients.

The researchers in the current study have opened the door to other types of studies and very eloquently presented their data to point to a transplant paradigm that may be beneficial to treatment after spinal cord injury by utilizing terminally differentiated cell types. Prolonged monitoring post four week transplant will need to be done to prove the lack of tumor formation and also allodynia associated with transplant models. Further, innervation of target tissues needs to be optimized as well. Matching HLA patient samples to the recipients may increase the complexity but this is promising research for the spinal cord injury field.

Does human umbilical cord blood derived mesenchymal stromal cells promote diabetic wound healing?

Evaluation of: You HJ, Namgoong S, Han SK *et al.* Wound-healing potential of human umbilical cord blood-derived mesenchymal stromal cells *in vitro* – a pilot study *Cytotherapy* 17(11), 1506–1513 (2015).

Diabetic chronic wound healing is a challenging field. There is no effective treatment available and any new therapeutics will raise a great interest to both patients, and healthcare providers [3].

Several studies have suggested that umbilical cord blood-derived stromal cells (UCB-MSC) promote diabetic wound healing [4,5]. Authors in this study further compared human UCB-MSC, healthy fibroblast (allogeneic fibroblast) and diabetic fibroblast in respect of cell proliferation, production of growth factors, and collagen synthesis. The results showed that hUCB-MSC was superior to diabetic fibroblast in both collagen synthesis and production of growth factor VEGF and FGF-2, and also superior to healthy fibroblast only in production of growth factor VEGF and FGF2, but not in the production of collagen.

Although it has been indicated that MSC promote wound healing, including diabetic wounds, the underlying mechanisms are still not fully understood. Disorder of angiogenesis in diabetes is considered one of the major factors responsible for unhealed wounds. Furthermore, studies have suggested that restoration of angiogenesis by VEGF might be one of the necessary steps for diabetic wound healing [6–8]. This study demonstrated that hUCB-MSC produced higher level of VEGF and FGF2 than diabetic fibroblast, and will potentially promote better angiogenesis. Tissue granulation is another major step towards wound healing, which requires collagen deposition. This study showed that hUCB-MSC produced higher quantities of collagen than diabetic fibroblast, suggesting hUCB-MSC

might accelerate diabetic wound healing by promoting collagen production as well. Interestingly enough, this study showed no difference of cell proliferation between three study groups. Other studies have demonstrated the impaired cell proliferation of diabetic fibroblast [9,10]. Nevertheless, this study raises a possibility that hUBC-MSC might accelerate the healing of diabetic wounds through VEGF- and FGF2-mediated angiogenesis and collagen production.

However, because hUBC-MSC will be allogeneic application when treating diabetic wounds, potential short lived allogeneic hUBC-MSC may not reproduce the result as the authors showed in this *in vitro* study. It is necessary to confirm the observation with *in vivo*

studies in order to claim the therapeutic functions of hUBC-MSC. Moreover, the immune modulatory effects of hUBC-MSC might need to be evaluated in diabetic wound healing.

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