International Journal of Infectious Diseases 32 (2015) 32–38

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Cellular therapy in Tuberculosis

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ARTICLE INFO

Article history:
Received 1 December 2014
Received in revised form 16 January 2015
Accepted 16 January 2015

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Tuberculosis
MDR-TB
host directed therapy
M.tuberculosis
inflammation
Mesenchymal stromal cells
T-cells
cancer
HDT

SUMMARY

Cellular therapy now offer promise of potential adjunct therapeutic options for treatment of drug-resistant tuberculosis (TB). We review here the role of Mesenchymal stromal cells, (MSCs), as well as other immune effector cells in the therapy of infectious diseases with a focus on TB. MSCs represent a population of tissue-resident non-hematopoietic adult progenitor cells which home into injured tissues increase the proliferative potential of broncho-alveolar stem cells and restore lung epithelium. MSCs have been shown to be immune-modulatory and anti-inflammatory mediated via cell-cell contacts as well as soluble factors. We discuss the functional profile of MSCs and their potential use for adjunct cellular therapy of multi-drug resistant TB, with the aim of limiting tissue damage, and to convert unproductive inflammatory responses into effective anti-pathogen directed immune responses. Adjunct cellular therapy could potentially offer salvage therapy options for patients with drug-resistant TB, increase clinically relevant anti-*M.tuberculosis* directed immune responses and possibly shorten the duration of anti-TB therapy.

1. Mesenchymal Stromal Cells

Mesenchymal stromal cells (MSCs) represent a population of tissue-resident non-hematopoietic adult progenitor cells, originally identified in the bone marrow,[1] and subsequently in a number of other organs.[2,3] MSCs were identified in the 1970s from cellular suspensions from spleen and bone marrow by their capacity to adhere to plastic – which is still the standard form for culturing MSCs. MSCs are able to form colonies from single cells (explanted *ex vivo*), have fibroblast-like appearance and capacity to differentiate into fat, cartilage and bone. Their function in bone marrow is to facilitate haematopoiesis for expansion of hematopoietic and embryonic stem cells,[4,5] thus may play a role in stimulating cell growth and organization in adult organ tissues.[2] MSCs have been shown to increase the proliferative potential of the so-called bronchoalveolar stem cells[6] and to restore lung epithelium via the transfer of mitochondria to other cells.[7,8] MSCs are defined by CD105, CD90 and CD73 expression and negative for CD45, CD34 and CD14.[9,10] More recent studies show that isolation of MSC from patients with underlying diseases may lead to different phenotypes, mantaining CD105, CD90 and CD73 expression. Bone-marrow derived MSCs may thus represent a mixture of different MSC populations[11] as has also been shown to be true for MSC from lung tissue. Sabatini showed in 2005[3] that a plastic adherent cell population exists in human lungs isolated via BAL[12] or via tissue digestion.[13] These cells are either long-lived or – not mutually exclusive – have the capacity for renewal. Given the fact that MSCs support human stem cells in the bone marrow, as well as bronchoalveolar stem cells, it is most likely that these functions

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http://dx.doi.org/10.1016/j.ijid.2015.01.016

may also exist in adult life - and that enrichment of MSCs into damaged lung tissue may aid to re-organize tissue and facilitate healing of chronic, unproductive inflammation associated with *Mcyobacterium tuberculosis (Mtb)* infection.

MSCs are believed to be facilitators of organ homeostasis and tissue repair following infection, neoplasms, damage and 'trauma' in general. MSCs are key cells in connective tissue hierarchy of organs, including the lungs. The roles of MSCs in the lung have recently been extensively reviewed by Sinclair and coworkers.[14] MSCs have been shown to be immune-modulatory, anti-inflammatory and immune-suppressive and most studies have looked at these effects in the allogeneic setting. *In vitro*, MSCs may decrease immune effector functions and aid to expand regulatory T-cells. Both cell-cell contacts as well as soluble factors could be mediating these effects[15,16] particularly on precursor and memory T-cell subpopulations.[16–18] Cell-cell contact appears to be important for expansion of Treg cells, defined by the CD4+CD25high, Foxp+ phenotype.[19,20] The functions of MSCs may be diverse and dictated by the immune-environment; thus making it difficult to predict how MSC will work in patients with lung tissue filled with live *Mtb* bacilli. For instance, co-culture of MSCs with PBMCs leads to prostaglandin E_2 (PGE_2) production in MSCs,[21] PGE_2 and COX2 are increased in the presence of type I interferons and/or TNFα suggesting that the effect of MSCs is influenced by the local cytokine milieu.[15,22]

The production of PGE_2[4,23] from MSCs may be particularly important in balancing unproductive inflammation in TB: High type I interferon levels affect TB disease outcome, increases tissue damage and subsequently increased *Mtb* proliferation. PGE_2 balances the inflammatory cytokines IL-1 and type-I interferons in individuals with latent TB; modulation of this host immune response axis has proven to be effective in preventing death in *Mtb*-infected mice. Studies in mice and analysis of *ex vivo* material from patients with TB demonstrated that IL-1 induces PGE_2 and suppresses type I interferons linked with clinical TB outcome.[24]

Environmental factors (metabolic programming), e.g. oxygen levels, have also been shown to influence the differentiation of MSCs into articular cartilage or epiphyseal cartilage.[25] Of note, more recent studies suggest that MSC may not only differentiate into fat, cartilage or bone, but also into bronchial epithelium, renal epithelium, neuronal tissue as well as cardiomyocytes. This is reflected in a number of studies using MSCs for non-mesenchymal tissues including brain, heart, and kidney diseases.[26] Several studies have now shown that clinical efficacy is not directly related to successful expansion and the level of MSC engraftment, yet to other factors (paracrine), driven by MSCs, which are yet to be identified. One of the aspects of MSCs is the polarization into pro- or anti-inflammatory cells, which appears to be triggered, at least in part, via TLRs. MSCs express TLR3 and TLR4. TLR3-agonists appear to polarize MSC to immune-suppression, whereas TLR4 stimulation leads to immune-stimulation of MSCs.[27] Several components of *Mtb* signal via TLRs and the local effect of MSC - in combination with the cytokine milieu and the TLRs – will contribute to the *Mtb* edited phenotype. One of the factors in *Mtb* infection is lung destruction via fibrosis and collagen synthesis, which is – in part – a TGFβ driven process. We showed that non-human primates that survive longer after *Mtb* challenge have a typical immune phenotype in their lungs, defined by less fibrosis, decreased TGFβ production and increased IL-7 and IL-17 production.[28] Of interest, TGFβ production has been shown to be repressed in TLR3-edited, yet not in TLR4-stimulated MSCs;[27] TLR3-primed MSCs showed up to the 80% reduced TGFβ production, which is mediated via TLR3-induced modulation of TGFβ – downstream effectors SMAD3 and SMAD7; TLR3 versus TLR4 stimulated MSCs also show differential IDO and PGE_2 production, which also underlines the local immune-editing milieu of *Mtb* infected tissues. PGE_2 converts macrophages into an IL-10 – producing phenotype. Immunomodulatory properties include the production of IL-1 receptor antagonists and the TSG-6 protein (anti-inflammatory protein TNFα stimulated gene protein 6).[29]

A number of clinical trials using MSCs as immune-modulatory agents or as stimulators for tissue generation have been reported. MSCs are being used for corticosteroid-resistant Graft versus Host Disease (GVHD) (*i.e.* inflammatory reactions after hematopoietic stem cell transplantation), and for treatment of other autoimmune diseases (Multiple Sclerosis, Crohn's disease etc.).[30,31] Sinclair and colleagues[14] in their phase I clinical study of MSC infusions in an allogeneic setting established safety of the allogeneic MSC infusion, with 2×10^6 cells / kg i.v. twice weekly for two weeks. The aim was to offer MSC for treatment of complications after lung transplantation as well as for the treatment of idiopathic lung fibrosis (www.clinicaltrial.gov). Another study evaluated the intra-tracheal administration of umbilical cord derived MSCs in children with bronchopulmonary dysplasia (www.clinicaltrials.gov/ct2/show/NCT01297205).

2. MSCs and infection

MSCs are susceptible to infection by several intracelullar pathogens such as *Mtb*, Influenza virus[30] and Herpesvirus-6 infection.[32] Conversely, MSCs have been shown to improve survival[33] in bacterial infections of mice which supports the concept as stated above that organ-damaging cascades in infections can be curbed with MSC treatment:[34] MSCs reduce inflammation–associated lung damage.[35,36] The safety of MSC therapy has recently been extensively reviewed by Lalu and coworkers.[37] Other beneficial effects may be the production of exosomes and microvesicles from MSCs which has been studied in the interaction of MSC and cancer cells,[38] but not in the context of MSC and pathogens. This is also a potential new area of investigation: if the signalling proteins and miRNA in the exosomes and microvesicles can be identified, potentially the cell therapy infusions can be obviated to the far simpler protein/miRNA infusions – if exosomal delivery of signals and proteins turns out to be biologically and clinically relevant in infections. Nauta and Fibbe reviewed the immunomodulatory properties of MSCs and showed the impact of MSCs on T-cell functions, including cytotoxicity; on dendritic cell functions (impaired CD83 and HLA-DR expression); B-cell function and NK-cells, defined by proliferation and cytotoxicity.[39] The type and severity of adverse effects may differ based on patient populations and the underlying disease, as well as the MSC characteristics used for expansion and subsequent therapy. A meta-analysis of the randomised clinical trials examining autologous and allogeneic MSC therapy in patients, searching MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials (till June 2011) did not detect associations between infusion toxicity, organ systemic complications, infections, death or malignancies. Whilst an association was identified with MSCs and transient fever, the application of MSC was found to be safe in 36 clinical studies. In addition, we have shown that MSC application in patients with MDR and XDR TB is safe.[40]

A recent publication has addressed the increasing use of MSC in the treatment of acute and chronic graft versus host disease (GVHD) in transplant patients with immunomodulatory effects and have suggested more prospective randomized controlled trials for optimisation of the MSC therapy.[31] Another recent report from the NIH clinical centre using third-party early passage (up to passage 3) MSCs infused at 2×10^6 MSCs/kg body weight IV weekly for 3 doses in a phase I clinical trial for patients with steroid-refractory GVHD following post-transplant complications established safety as well as significant rapid clinical responses and biomarker normalisation among the majority of the study participants. The study observed positive outcomes in patients with a relatively intact immune system with higher absolute lymphocyte counts and favourable cytokine and T cell phenotype patterns.[41]