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Scientific Letter

Mesenchymal stem cells as broad application therapeutics

Background

Stem cells are immature cells that have the potential to divide into various mature cell types. They have other properties which make them potentially useful as therapeutic tools in the treatment of disease or injury. Stem cells are of two main types, embryonic and adult non-embryonic stem cells. Embryonic stem cells are formed when a newly fertilized human egg begins to divide, and can form all cell types found in the body. Non-embryonic adult stem cells are found in tissue around the body, and have more limited abilities to form other cell types. Adult non-embryonic stem cells called mesenchymal stromal cells (MSC) are being developed as a platform for next generation medical countermeasures (MCMs) against biological and chemical agents and are being evaluated for use as therapies for traumatic injuries in military medicine.

Mesenchymal stromal cells (MSC, also called mesenchymal stem cells) are non-embryonic cells which can naturally form some different cell types (Figure 1), and have unique useful properties. In the body, the natural population of these cells is usually found within normal tissues, doing not very much. During an inflammatory process (such as injury, infection, trauma), these stem cells mobilize in response to chemical signals from the affected tissue, home in to the affected area, and begin to express molecules which aid in inflammation control, immune system modulation, and tissue healing. MSCs can be readily modified in various ways to improve their utility for specific therapeutic needs.

Mesenchymal stromal cells are also immune privileged; which means that mesenchymal stem cells can be transplanted from any individual to any other individual without the need for genetic and immunological donor matching. This makes MSCs a potentially powerful therapeutic tool, because they can be grown in a laboratory to large numbers, then implanted into patients to assist in disease treatment and recovery.

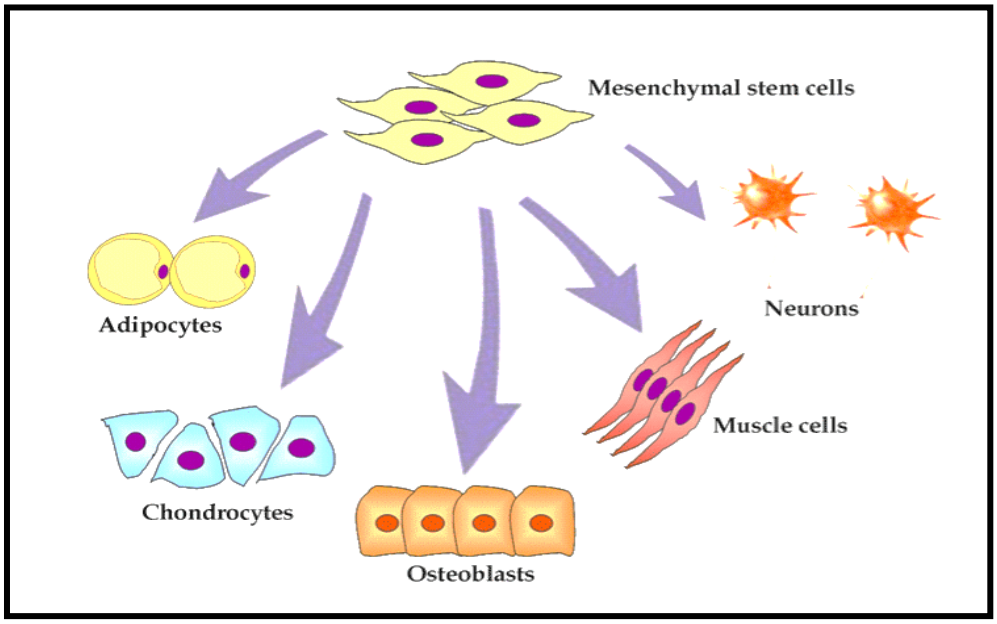

Figure 1: Normal fate of MSC, adapted from omicsonline.org, 2015.

MSCs are well characterized as to safety, and are amenable to regulatory-compliant manufacturing and stockpiling. The MSC platform is transformative because it enables novel therapies against multiple agents, and can improve the usefulness of ***already developed*** medical countermeasures that have limited clinical value due to partial activity, rapid immune degradation, or limited efficacy. They may also be valuable as adjuncts to treatment of traumatic casualties (e.g. sepsis due to blast injury). MSC can be used either as native cells, modified cells with enhanced properties, or as sterile bioactive extracts from cultured cells. For example, modified cells can express specific antibodies, as prolonged therapeutic or preventive tools against difficult to treat viral infection. Cell or culture media extracts from stimulated MSC can be purified away from the cells, and prepared as a fieldable freeze-dried powder for first-aid treatment of wounds or burns.

The particular mesenchymal stem cells of interest to Defence Research and Development Canada are supplied by a Canadian industrial collaborator (Tissue Regeneration Therapeutics, Toronto), and are also called human umbilical cord perivascular cells (HUCPVC). These are non-embryonic adult-type stem cells which come from tissue usually discarded as waste after normal births. They do not represent ethical or source supply issues.

The work being undertaken involves applications of MSC as treatment for injury and infection, and as medical countermeasures to chemical agents, with particular emphasis on difficult therapeutic issues of interest to the Canadian Armed Forces. Within the main areas of effort discussed in this document are continuing studies in basic biology of mesenchymal stem cells, including the effects of long term culture and cryopreservation on viability and biological activity.

Successes to date

In collaboration with the industrial partners, the ability to culture mesenchymal stem cells to large numbers, and cryopreserve them as a rapidly deployable stockpile has been demonstrated. Proof of concept of the expression of antibodies as an enhanced delivery platform versus intravenous infusion of antibody in solution has been completed, and will be

further developed towards a preclinical state. Cell-based therapeutics are typically administered by intra-venous infusion, a route which is not optimal for military purposes in austere environments. We have shown that intramuscular injection of MSC (a fieldable technique as opposed to intravenous infusion) allows for survival and persistence of stem cells expressing therapeutic molecules (Braid et al., 2015). To generate a product suitable for field deployment, we have tested the HUCPVC platform using an intra-muscular administration route which is simple, robust, and highly amenable to use in the field. Stockpiling and field-useable intramuscular injection are important milestones, without which the eventual utility of stem cells as far forward medical tools would be limited.

Current areas of effort

1) MSC-mediated antibodies as treatment for viral infection

Viral infections remain a difficult to treat problem. Recent experience with Ebola in West Africa has shown that preparations of human antibodies can be used to good effect in some viral diseases. We recently demonstrated the potential of the HUCPVC platform for *in vivo* production of an antibody against infection with equine encephalitis virus (VEEV; Braid et al., 2015). DRDC Suffield has developed and patented this antibody against VEEV, which is protective against this select biological threat agent infection. Unfortunately, duration of protection by the infused antibody is limited by the natural degradation by host immune and metabolic processes.

However, mice pretreated with MSCs engineered to express this antibody against Venezuelan equine encephalitis virus (VEEV), were better protected, and for longer duration, than mice pre-treated with the purified antibody alone. The protection persists for a long period, due to the continued expression of the antibody from the implanted MSCs. Antibody expression from modified MSCs was detected more than 175 days after intra-muscular administration. Thus, efficacy of the antibody therapy was improved by changing the way in which the antibody was delivered. Continuing work in this area will be to translate this R&D product at Technology Readiness Level 4, to a preclinical state suitable for Phase 1 clinical trial.

2) Human butyrylcholinesterase as an antidote for organophosphate exposure

Organophosphate (OP) poisoning occurs through inhalation or skin exposure to pesticides and weaponized nerve agents, which inhibit acetylcholinesterase (AChE). The resulting hyper-stimulation of nerve activity manifests as seizures, convulsions, and death. Butyrylcholinesterase (BChE) is found at low levels in human serum; its similar functionality to AChE confers potential as a therapeutic against OP intoxication. In animal models of OP poisoning, BChE is effective both as a pre-exposure prophylactic and as a post-exposure treatment, although efficacy is short-lived, for the same reasons as infused antibody therapies are time limited. Phase 1 clinical trials have found no toxicity associated with high or repeated doses of BChE. However, clinically useful quantities of functional BChE developed from expired human plasma, transgenic goats, tobacco plants and insects, have all proven problematic and costly.

We are developing MSCs as a BChE delivery system for protection against, and treatment after, exposure to OPs. Mesenchymal stem cells naturally secrete BChE; as such, they have an innate capacity to produce BChE comparable to the benchmark human plasma form (among others, Elman et al., 2014). We have established that MSCs readily express a human BChE genetic construct, and subsequently increase their output of active BChE. We propose to

generate a pharmacological profile of circulating BChE in mice receiving an intra-muscular treatment of BChE-gene modified MSCs, to determine how quickly the implanted cells can generate protective levels of BChE, and how long such levels can be sustained. Once it has been shown that BChE levels are enhanced by the MSC-BChE implantation, a demonstration of efficacy against a known chemical agent (Sarin) will complete the proof of concept.

3) MSCs to mitigate microbial infection

The third approach exploits the natural immune-modulatory properties of mesenchymal stem cells to improve efficacy and expand the treatment window for traditional therapies by modulating the recipient's immune response to infectious agents. The biothreat agent *Francisella tularensis* causes tularemia, with a low-inflammatory replicative state followed by a hyper-inflammatory state, resulting in local and systemic tissue damage. Without early and appropriate antibiotic treatment, disease progression leads to respiratory failure and sepsis. Antibiotic treatment can be most effective when initiated prior to symptom onset, but is frequently of long duration with significant side-effects.

MSCs can suppress immune effectors, via intercellular signaling, during the hyper-immune response, and thus lessen the damaging effects of the pro-inflammatory phase, preserve organ function, and improve the efficacy of antibiotic treatment (Gorbunov et al., 2013). We will test whether natural MSCs can attenuate the hyper-immune response and extend the treatment window of fully virulent *F. tularensis* infection. We will test whether these effects can be manipulated *in vitro*, prior to administration of MSCs, to further enhance their benefit. The long term goal of this work is the development of therapies that combine the natural immune-modulatory properties of the stem cell with a therapeutic genetic payload (described above), to improve existing treatment techniques.

4) Burn injury first response treatment

Flash burn and associated trauma due to explosion are challenging issues for forward casualty management. MSCs could be of use close to the time and point of injury, but are limited by storage and administration, which are currently, best done in at least a Role 3 situation. However, the healing properties of MSC are expressed by release of biomolecules from the cell into the host circulatory system (Arno et al., 2014). Release of these molecules can also be stimulated in the laboratory, into the medium in which the cells are grown. This medium can be separated from the living cells, dried, and used directly as a powder akin to sulfa antibiotics. In collaboration with an industrial partner, Aurora BioSolutions Ltd, the current effort will test whether the bioactive fraction from the cultured cells can be freeze-dried onto a field bandage, while retaining much of its wound healing properties. This device has the potential to change the treatment delay of forward injuries from hours down to minutes, and is highly compatible with austere low burden requirements.

Significance and future

Casualty care, both immediate and short term, remain challenges for deployed forces. Far forward injury treatment and mitigation, remain primary concerns. Stem cell treatments which can be pushed forward could reduce mortality and morbidity on the field.

Defence R&D Canada is interested in the clinical advancement of countermeasures to combat nerve agent poisoning. Recent intentional use of nerve agent in Syria is evidence of the continuing threat of chemical weapons use, and the need for advancement of countermeasures.

BChE is a well-characterized antidote for nerve agent poisoning, and has already been proven safe in Phase 1 clinical trials. However, adequate sources of biologically active BChE have remained elusive. The proposed research will examine the utility of a stem cell-based platform to produce sustained therapeutic BChE levels with a single intra-muscular dose. This product will have significant global value, for military and civilian health.

MSCs are actively being investigated as a novel treatment modality for sepsis. Sepsis is a frequent issue following trauma, and a hallmark of biothreat agent infection. The proposed work is important to the military because it focuses on agents or injuries that can cause substantial impact to operations and have significant mortality and morbidity rates. Post- trauma infections can be very hard to treat effectively without adverse impact on the individual being treated. Expanding the window of effective treatment and lessening the disease impact would be beneficial to maintaining the capability of the war fighter. Moreover, this work will be carried out using DRDC – Suffield Research Centre's highly characterized animal models of infection that are accepted by Health Canada and the Food and Drug Administration (FDA) for pre-clinical data.

The future uses of stem cell platforms in casualty care include tissue and organ repair and regeneration, possibly even limb repair. Development in such applications is a foreseeable future area of work, on the five to seven year horizon.

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Annex A Supplementary References

Table A.1: *Braid et al, 2015.*

Braid, L., Hu, W., Davies, John E., Davies and Les P. Nagata. 2015. Transfected mesenchymal cells as a prophylactic biomedical countermeasure to Venezuelan equine encephalitis virus exposure. Unpublished.

Abstract: Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne pathogen affecting humans and equines, and can be used in bio-warfare. In humans, VEEV causes a pathological spectrum including acute neurological encephalitis. No licensed vaccine or antiviral currently exists to combat VEEV infection in humans. Direct antibody administration (passive immunity) is an effective, but short-lived, means of providing immediate protection against a pathogen. We examined whether human umbilical cord perivascular cells (HUCPVCs), engineered with a transgene encoding a humanized VEEV-neutralizing antibody (anti-VEEV), could provide a renewable source of antibody protection in vivo. In mice, the anti-VEEV antibody had a half-life of 3.7 days, limiting protection to 2 or 3 days after administration. In contrast, modified HUCPVCs generated protective anti-VEEV serum titers for 21 to 38 days. At 109 days post-transplant, 10% of mice still had circulating anti-VEEV antibody. Importantly, mice were protected against exposure to a lethal dose of VEEV by a pre-treatment with modified HUCPVCs 24 hours or 10 days before exposure, demonstrating both rapid and prolonged immune protection. This study is the first to describe mesenchymal stromal cells as gene delivery vehicles for passive immune protection from a pathogen.