ImmunoTools *special* Award 2018

Barbara Fazekas, PhD
Postdoctoral Researcher

Regenerative Medicine Institute (REMEDI)
Biomedical Sciences Bldg, BMS-1020
First Floor South Research Area
Dangan, Galway, Ireland

The therapeutic potential of a novel human mesenchymal stromal cell preparation in sepsis-associated acute kidney injury

Sepsis is a common, severe medical condition associated with systemic inflammation and acute damage to multiple organs. The prognosis is particularly poor when acute kidney injury (AKI) occurs. Intravenously administered mesenchymal stromal (stem) cells (MSC) reportedly modulate systemic and localised inflammation in animal models and are in the early stages of clinical translation for sepsis. However, optimisation of MSC source, dose and mechanism of action within the kidney during sepsis-associated AKI has not been achieved.

In this 2-year postdoctoral research project, an established mouse model of polymicrobial sepsis will be applied to optimise the beneficial potential of a novel human MSC therapeutic product for sepsis-associated AKI and to better understand its in vivo mechanism of action. This project also aims to test if multiple dosing approaches ameliorate the severity of the disease.

The ultimate goal is to determine the potential for multiple doses of human bone marrow (BM) - and umbilical cord (UC)-derived MSC to modulate sepsis-associated acute kidney injury (AKI).

The central research questions are (a) What is the bio-distribution of BM-MSC and UC-MSC following intravenous administration in the setting of sepsis? (b) How is the severity of sepsis-associated AKI affected by multi dose intravenous administration of BM-MSC and UC-MSC? (c) What intra-renal immune and inflammatory pathways are modulated by multi-dose BM-MSC and UC-MSC in the setting of sepsis-induced AKI?

If these pre-clinical studies show efficacy with our stromal cell product, the generated data will be used for regulatory applications to plan first in human clinical trial with well-defined antibody-purified stromal cell therapy.

Profiling of intra-renal immune/inflammatory cells will be performed by multi-colour flow cytometric analysis of collagenase/DNase-digested kidney tissue. Other affected organs, such as lungs, spleen, liver and lymphoid nodes will be studied, as well. I plan to use the IT box provided by the ImmunoTools for multicolour flow cytometric phenotypic analysis that will require a combination of CD45, CD4, CD8 and TCR-β to identify T cells. Additional markers – CD19 or NK1.1 – will allow distinction of B cells, NK (Natural Killer) cells or NKT cells. Further antibodies will screen for known monocyte/macrophage markers such as CD11b, Ly6C and F4/80 and for discrimination of dendritic cells and neutrophils (CD11c, Ly6G). Many of these

antibodies are present in the IT box and are available with different fluorochrome tags, enabling multicolour detection.
Secondly, antibodies from the IT box may be used for functional studies. Co-cultures of human monocytes and primary human renal microvascular endothelial cells (RMEC) will be stimulated with pathogen-associated molecular patterns (PAMPs) in the presence or absence of hBM-MSC or hUC-MSC to analyse the MSC effects on innate immune cell/endothelial cell interactions. After culture, cells will be harvested, and RMEC and monocyte activation, phenotype and viability will be analysed by multi-colour flow cytometry using the above listed antibodies, while MSCs will be studied for migration ability by additional human markers such as CD44 or CXCR4 (chemokine receptor type 4).
Finally, cytokine release in serum isolated from whole blood could be measured through ImmuoTools ELISA kit for TNF-alpha and IL-6. The ImmunoTools *special* AWARD would prove to be a great asset to enable me to carry out this study.

ImmunoTools *special* AWARD for **Barbara Fazekas** includes 21 reagents

APC – conjugated anti-mouse CD4, CD8a, CD11b, CD19, CD45, NK cells

FITC - conjugated anti-mouse CD19, CD25, CD44, CD45, alpha/beta TCR, NK cells

PE - conjugated anti-mouse CD4, CD8a, CD11b, CD19, alpha/beta TCR

mouse ELISA set (for one 96 plate): mouse IL-6, mouse TNF-alpha

DETAILS more AWARDS