Mesenchymal Stromal Cells Reprogram Hematopoietic Stem Cells for Trained Immunity

Julie Ng, Anna Marneth, Alec Griffith, Daniel Younger, Sailaja Ghanta, Brandon Hancock, Fei Guo, Mark Perrella, and James Lederer
BWH and Harvard Medical School, Boston, MA, USA

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Paracrine factors from mesenchymal stromal cells mediate trained immunity by epigenetic and mTOR signaling pathway modifying effects on hematopoietic stem cells

Background and Introduction

- Novel prophylactic strategies are urgently needed to prevent opportunistic infections in immunocompromised individuals
- **Trained immunity**, or the functional reprogramming of innate immune cells to enhance the host immune response against a broad range of subsequent infections, is a promising approach to help restore immune function in immunocompromised people
- Previous findings showed that mesenchymal stromal cells (MSCs) preconditioned with a class A CpG oligodeoxynucleotide (CpG-ODN), a Toll-like receptor 9 (TLR9) agonist, can augment **emergency granulopoiesis** in a murine model of neutropenic sepsis
- Here, a chimeric mouse model was used to demonstrate that CpG-ODN preconditioned MSCs (CpG-MSCs) secrete paracrine factors that act on hematopoietic stem cells (HSCs), leaving them 'poised' to enhance emergency granulopoiesis months after transplantation

Methods

Chimeric mouse model to study MSC mediated neutrophil trained immunity

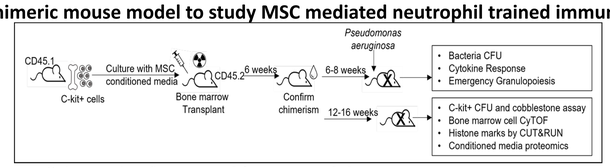

Figure: Schematic of **mouse chimera model**

- C-kit+ lineage- cells from the bone marrow of congenic CD45.1 mice were transferred to irradiated (4.5 Gy x 2) CD45.2 mice. Chimerism was confirmed by flow cytometry of blood samples
- The listed assays were performed in chimera mice that were either infected with *P. aeruginosa* in the lungs or un-infected as appropriate

Mouse lung infection model – Mice were anesthetized and infected in the lungs with *P. aeruginosa* (1-2 x 106 CFU) by inhalation of 40 μL bacteria.

Cobblestone-area forming cell (CAFC) assay – MS5 stromal cells were grown and co-cultured with 1 x 10^3 HSCs for 7-9 days develop myeloid cells

Colony forming unit assays – Bone marrow lin-/c-kit+ cells from chimeras were mixed in MethoCult and incubated for 9 days prior to scoring CFU-GM, -G, -M.

Signaling Mass Cytometry (CyTOF) – CAFC cells were stimulated for 1, 10, or 30 minutes with MSC conditioned media (CdM) or LPS, stabilized, then stained to detect phosphorylated signaling molecules in single cells.

CUT&RUN – CUT&RUN assays identified genes and signaling pathways affected by histone modification (H3K4me3). Sequencing data was analyzed to identify gene promoter sequences and signaling pathways.

Proteomic analysis – CdM was subjected to mass spectrometry. Proteins were identified by SEQUEST and the top 10 proteins were inputted into Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) to identify networks.

Results

1. Chimeric mice developed from CpG-MSCs or MSCs showed significantly higher bacterial clearance and increased neutrophil granulopoiesis following lung infection with *P. aeruginosa* than age/gender matched control mice.
2. CUT&RUN chromatin sequencing identified that MSC CdM leaves H3K4me3 histone marks in HSCs at genes involved in myelopoiesis and mTOR signaling persistence (validated by signaling CyTOF).
3. Both soluble factors and extracellular vesicles (EV) from MSCs mediate neutrophil trained immunity effects on HSCs.
4. Proteomics by mass spectrometry identified **soluble calreticulin** as a potential mediator of CpG-MSC paracrine activity on trained immunity.

Brigham and Women's Hospital
Harvard Medical School
https://ledererlab.bwh.harvard.edu/

FIGURES AND TABLES

Figure 1: 6-Week Chimerism and Blood Immune Cell Subset Percentages

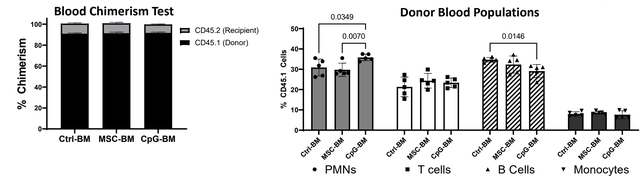

Figure 2: Lung *P. aeruginosa* Infection Responses in Chimeras: Chimeras developed from HSCs exposed to MSC and CpG-MSC conditioned media demonstrate heightened anti-microbial immunity

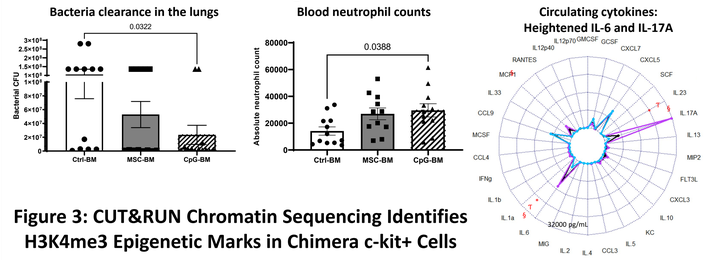

Figure 3: CUT&RUN Chromatin Sequencing Identifies H3K4me3 Epigenetic Marks in Chimera c-kit+ Cells

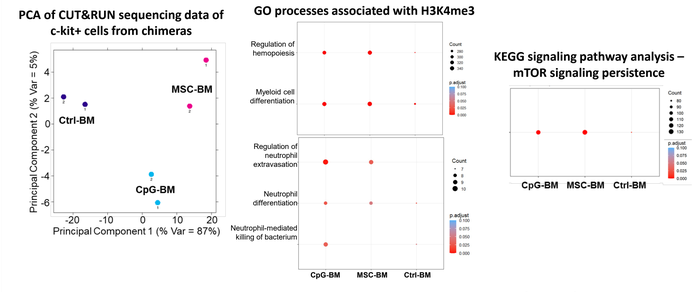

Figure 4: *In Vitro* Cobblestone-Area Forming Cell Assay (CAFC) Studies on Neutrophil Signaling Response to CpG-MSC CdM

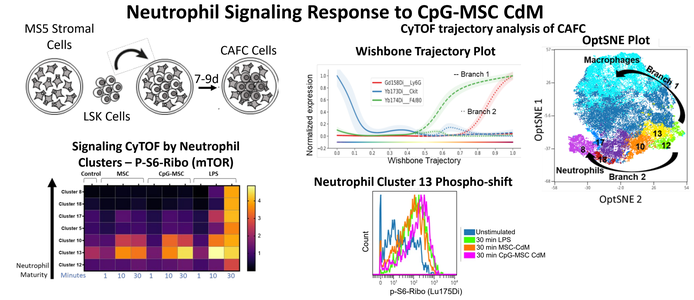

Figure 5: Paracrine Factors from CpG-MSCs that Induce Trained Immunity

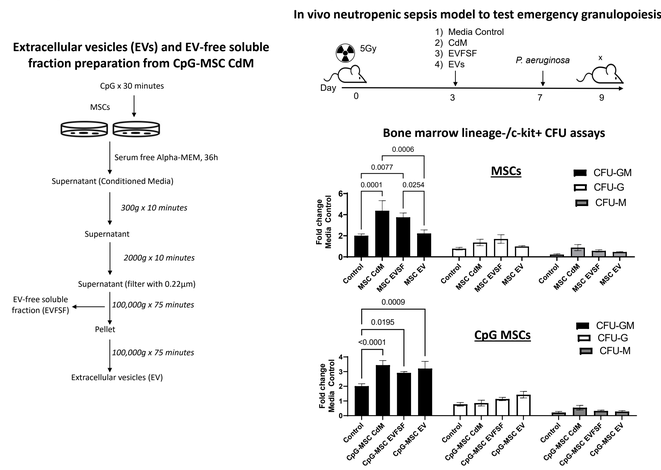

Figure 6: Mass Spectrometry Proteomics of CpG-MSC Conditioned Media