The adaptive response of mesenchymal stem cells to danger signals regulate neutrophil activation in cutaneous wounds

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Background and Hypothesis

MSCs are most likely endowed with the capacity to sense environmental cues like infection and to generate an integrated adaptive response in the interest of tissue protection. So far it is, however, largely unexplored how MSCs sense their environment and how they mount an adaptive response to shape the function and activation state of distinct immune cells in particular neutrophils during tissue injury.

Previously, we have shown that MSCs suppress neutrophil activation by dampening their oxidative burst, the release of reactive oxygen species (ROS), proteolytic enzymes and by phagocytosis of activated neutrophils leading to scar reduced tissue repair. In the present study, we wished to investigate how MSCs adaptively regulate neutrophils functions under conditions of wound infection, where MSC suppression of neutrophil functions would rather be detrimental.

Figure 1. Schematic representation of the hypothesis. (a) Neutrophil functions to clear bacteria by phagocytosis, oxidative stress and proteolytic degradation. (b) MSC may exhibit immunosuppressive effect on neutrophil activation. (c) MSC may build an adaptive response on neutrophil activation in infectious microenvironment (d) TLR-silenced-MSCs may not be able to exhibit adaptive response on neutrophil activation in infectious microenvironment.

Figure 2. h-ADMSCs fulfill minimal criteria of stem cells. (a) The expression profile of surface markers of h-ADMSCs was analyzed by flow cytometry. AD-MSCs expressed specific markers and do not express other markers. (b) h-ADMSCs were cultured in adipogenic, osteogenic and chondrogenic induction media and subjected to Oil-Red-O staining indicative of adipogenic, Alizarin Red staining indicative of osteogenic and aggrecan staining indicative of chondrogenic differentiation, respectively. Scale bar: 50µm.

Figure 3. LPS-treated h-ADMSCs augment neutrophil activation resulting in an increased NET and ROS production. (a) h-ADMSCs and activated neutrophils were co-cultured with an increasing concentrations of LPS in the presence of PMA. There is an increase in NET formation with an increasing concentrations of LPS. Scale bars: 100µm. (b) Graph show quantification of the NET bound elastase enzyme. A significant increase in NET formation occurred in LPS treated h-ADMSCs groups compared to non LPS treated group. *** $P \leq 0.001$, $n=3$. (c) h-ADMSCs were co-cultured with neutrophils and treated without and with LPS in increasing concentrations. It result in high production of ROS suggesting an adaptive role of h-ADMSCs. *** $P \leq 0.001$, $n=3$.

Figure 4. LPS treated h-ADMSCs activate MyD88 and IL-8 expression in the TLR4 signaling pathway. h-ADMSCs were treated with LPS. Expressions of the cytoplasmic protein MyD88 and IL-8 was assessed by western blot analysis. Densitometry assessment of MyD88 and IL-8 show a significant increase in MyD88 and IL-8 expression upon LPS exposure. Actin served as loading control. *** $P \leq 0.001$, $n=3$.

Figure 5. LPS-treated TLR4-silenced-ADMSCs do not augment neutrophil activation resulting in decreased NET production. (a) TLR4 silenced and non-silenced h-ADMSCs were co-cultured with neutrophils and treated with LPS. TLR4 silenced, LPS treated h-ADMSC showed reduced NET formation. Scale bars: 100µm. (b) Quantitative assessment of NET bound elastase enzyme by means of a specific ELISA showed a significant reduction of NET bound elastase in TLR4 silenced h-ADMSCs as opposed to high NET bound elastase in non-silenced h-ADMSCs co-cultured with activated neutrophils. *** $P \leq 0.001$, $n=3$.

Figure 6. LPS treatment significantly altered transcriptome of h-ADMSCs. (a) RNAseq analysis of the top 50 up- and downregulated genes after 6 and 24h of LPS treatment showed upregulation of IL-8, IL-1β, CSF2, C3, CXCL6 and ICAM-1 and downregulation of IRAK3 and RGS7. Blue bars and red bar in heat-map show reduced and high expression of genes, respectively. (b) RT-PCR results show upregulation of IL-β and downregulation of RGS-7 after 24h of LPS treatment *** $P \leq 0.001$, $n=3$. (c) ELISA results showed significant increase in IL-8 expression after 6 and 24h of LPS treatment, while CXCL6 is upregulated after 24h of LPS treatment. Western blot analysis showed upregulation of CXCL5, IL-1 β, TLR4 and IL-6 expressions upon LPS treatment. *** $P \leq 0.001$, $n=3$.

Figure 7. NET formation in acute wounds treated with LPS primed h-ADMSCs. h-ADMSCs with and without LPS treatment were injected into wound margins in mice. Immunostaining depicts enhanced NET formation in the LPS treated h-ADMSCs group 24h after wounding compared to reduced NET formation in h-ADMSCs which had not been exposed to LPS. Scale bar: 20µm.

Conclusions

Figure 8. Sensing and shaping the neighborhood. Graphical summary, depicting the molecules involved in sensing, signaling and raising an adaptive effector response in h-ADMSCs to shape neutrophil recruitment and activation.

Future perspective

This study can be helpful to disclose complex functional interactions between MSC and neutrophils upon bacterial challenge and provide evidence for an adaptive role of MSC in the wound microenvironment in the early phase of immune response. In a long run, immunomodulatory properties of MSC can be utilized for therapeutic purpose as for wound healing, sepsis and endotoxin shock. Our data may even hold promise to be therapeutically exploited for the benefit of patients with difficult-to-treat and/or infected wounds.

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