

COMPLEMENT MODULATES MESENCHYMAL STEM CELL DIFFERENTIATION

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Introduction: Mesenchymal stem cells (MSCs) are adult multipotent cells which can reciprocally differentiate into adipocytes or osteoblasts. C3 is pivotal in the complement system which is a central part of innate immunity. Although previous studies have connected the innate immune system with MSC differentiation, the relationship between complement and MSC differentiation remained completely unknown.

Methods: MSCs were isolated from femur bone marrows of 6 week old female WT, C3^{-/-} and C5aR^{-/-}C3aR^{-/-} mice (all on C57BL/6 background) using MesenCult Media (Stemcell Inc, Canada) according to the manufacturer's protocol. The isolated MSCs were tested for expression of typical MSC cell markers and different complement receptors using respective monoclonal antibodies followed by flow cytometry analysis. RT-PCR was performed to detect complement component C3, C5, factor B and factor D transcripts using total RNA isolated from WT MSCs and ELISA was used to compare IL-6 levels in supernatants from WT, C3^{-/-} and C5aR^{-/-}C3aR^{-/-} MSCs cultures. For differentiation studies, same passage numbers of WT and C3^{-/-} MSCs were subjected to adipocytic and osteoblastic differentiation conditions for 21 days followed by Red Oil O staining for adipocytes and Alizarin Red S staining for osteoblasts. The relative expression levels of PPAR γ and Runx2 in the differentiated WT and C3^{-/-} MSCs were assessed by qRT-PCR.

Results: All isolated MSCs are negative for CD34, CD45 and CD11b while positive for CD29 and CD117. C5aR and C3aR are present on WT MSCs but the other complement receptors i.e. CR1, CR2, CR3 and CR4 are not detectable. MSCs locally produce C3, C5, factor B and factor D, which are essential factors for C5a and C3a production through the alternative pathway complement activation. MSCs from C3^{-/-} or C5aR^{-/-}C3aR^{-/-} mice produce ~5 fold less IL-6 than MSCs from WT mice, suggesting that IL-6 production in MSCs is regulated by complement through C5aR and/or C3aR signaling. Under differentiation conditions, C3^{-/-} MSCs exhibit significantly increased adipocyte generation and decreased osteoblast generation compared to WT.

Accordingly, they possess 4-8 fold higher expression levels of PPAR γ and 9-15 fold lower expression levels of Runx2 than MSCs from WT mice. C5aR^{-/-}C3aR^{-/-} MSCs exhibit enhanced adipogenesis and decreased osteogenesis similar to C3^{-/-} MSCs.

Conclusion: Complement, locally produced by MSCs, is integrally involved in MSC differentiation and the primary underlying mechanism could be the autocrine C5aR and/or C3aR signal regulated MSC IL-6 production. These results indicate that manipulating complement activation could be a new strategy to accelerate osteoblast generation in different clinical settings.