

MESENCHYMAL STEM CELLS INHIBIT DENDRITIC CELL DIFFERENTIATION AND FUNCTION BY PREVENTING ENTRY INTO THE CELL CYCLE

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Summary

The immunosuppressive effect of mesenchymal stem cells (MSC) has great potential for therapeutic use, although it has not been fully characterised. This study evaluates the immunosuppressive effect of MSC on dendritic cells (DC) differentiation. DC were generated from purified human monocytes and co-cultured in the presence or absence of MSC. Newly formed DC were collected from the cultures on day 4 and 6 for cell counting, immunophenotyping and functional assays. The results indicate that, MSC impair differentiation of DC by arresting them in G₀ phase of cell cycle.

Introduction

Mesenchymal stem cells (MSC) are non-haemopoietic stem cells that reside in bone marrow and characterised by their higher self-renewal ability and differentiation capability into tissues of mesenchymal origin (osteocytes, chondrocytes, and adipocytes) ^[1]. Physiologically, MSC constitute a haemopoietic niche in the bone marrow microenvironment and regulate the survival, self-renewal, migration, and differentiation of haemopoietic stem cells (HSC) ^[2]. Besides, MSC have also been shown to regulate early T cell lymphopoiesis in the bone marrow ^[3] as well as T cell development in thymus ^[4]. We have shown that MSC induce in T cells a state 'split tolerance' whereby activated T cells are arrested in the G₀/G₁ phase of cell cycle as consequence of cyclin D2 inhibition. This study aimed to investigate the similar effects on DC.

Materials and Methods

MSC were generated from human adult bone marrow (BM) aspiration. Briefly, mononuclear cells from BM cultured using commercially available media. After two weeks, adherent cells were detached and passaged. Monocytes were isolated from buffy coats using CD14-conjugated microbeads. Purified monocytes (5x10⁶/well) were cultured in DC differentiating medium containing GM-CSF and IL-4 in 6 well plates in the presence or absence of MSC at 1:10 MSC:monocyte ratio. Cells were collected at day 4 or 6 for cell counting, immunophenotyping, cell cycle analysis and functional study. Irradiated monocytes (60 Gy) were used as control.

Result

In the presence of MSC the number DC generated from monocytes was reduced of 50% as compared to the control cultures. Furthermore, the percentage of CD14⁺ cells harvested from MSC culture was significantly higher as compared to controls (85% vs. 12% respectively) and the expression of HLA-DR and CD11c was much reduced. At the cell cycle analysis, in the presence of MSC 77% of DC were in G₀ phase and only 20% of the cells entered the protein synthesis phase G₁. Conversely, DC generated in the absence of MSC were mostly in G₁ phase (75%) and only 23% of DC resting at G₀. Accordingly, significantly fewer DC entered S and M phases in the

presence of MSC. The DC generated in the presence of MSC were tested for their ability to mediate antigen presenting function for CD4⁺ allogeneic T cells. When DC generated from MSC coculture were used only 30% of CD4⁺ T cells proliferation was observed as compared to control DC. To identify the mechanism at which DC cell cycle is arrested by MSC we evaluated the expression of the principal molecules involved in cell cycle regulation. The western blot analysis revealed that MSC downregulate the expression of cyclin D2 and D3 which are regulators of cell cycle check points.

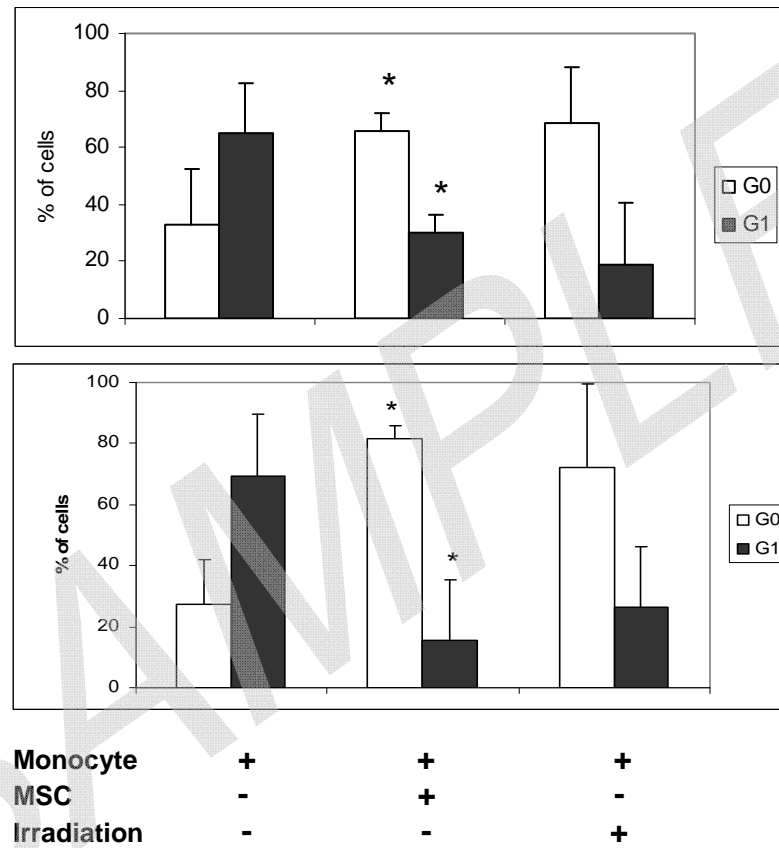


Fig.1: MSC arrest the DC at G₀ phase of cell cycle
 *significant at 0.05 level

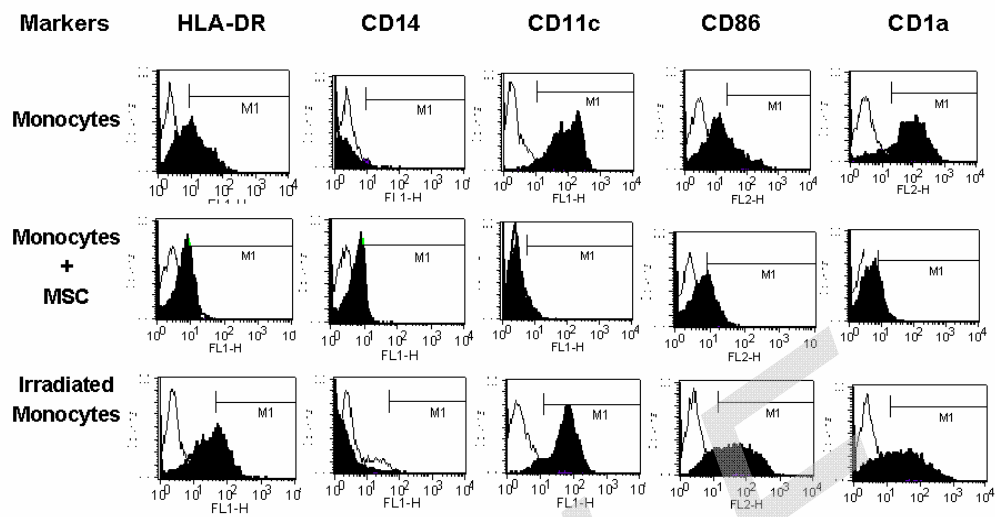


Fig.2: MSC inhibit the differentiation of monocytes into DC

Discussion

In conclusion, our data indicate that impairment of DC differentiation by MSC is a consequence of cell cycle arrest especially by cyclin D2 downregulation. This finding suggests that this is the fundamental mechanism accounting for the immunosuppressive activity of MSC.

Reference

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