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MODULATION OF THE IMMUNOSUPPRESSIVE FUNCTIONS OF *EX VIVO* GENERATED MURINE MYELOID-DERIVED SUPPRESSOR CELLS WITH CYTOKINES AND TUMOR CONDITIONED MEDIUM *

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Abstract

Myeloid-derived suppressor cells (MDSCs) are immune cells linked to immunosuppression in cancer, inflammation, autoimmune diseases, and transplantation. They comprise polymorphonuclear (PMN-MDSCs) and monocytic (M-MDSCs) subsets resembling neutrophils and monocytes. MDSCs suppress other immune cells, primarily T cells.

A comprehensive understanding of MDSC biology and mechanisms of action, including the identification of novel markers and therapeutic targets, is essential for effectively overcoming tumor-induced immunosuppression and developing efficacious interventions for inflammatory and autoimmune diseases. However, the absence of a standardized *ex vivo* MDSC generation protocol represents a significant obstacle to comprehensive investigations of these cells, underscoring the urgent need for the development of such methodologies within the field of immunology.

In this work, we compared six different protocols for generating MDSCs *ex vivo*. Murine bone marrow precursors were used to generate MDSCs *ex vivo*. The protocols involved the use of granulocyte-macrophage colony-stimulating factor (GM-CSF), either solely or in conjunction with granulocyte colony-stimulating factor (G-CSF) or interleukin-6 (IL-6), with or without the addition of tumor conditioned medium (TCM), substances known to promote MDSC expansion and activation *in vivo* [1]. The resulting MDSCs were characterized in terms of their morphology, phenotype, immunosuppressive function on T cell proliferation, and gene expression of crucial immunosuppressive factors.

Analysis revealed that all experimental protocols yielded approximately 75% MDSC, comprising 25% monocytic and 50% polymorphonuclear MDSCs. The results showed that the combination of GM-CSF with IL-6 or G-CSF increased the M-MDSC and PMN-MDSC yields, respectively, compared to GM-CSF alone. Protocols incorporating IL-6 was found to significantly reduce MDSC maturation and differentiation while increasing the expression of immunosuppressive factors. All generated MDSCs effectively suppressed T cell proliferation capacity *ex vivo*, with GM-CSF and G-CSF+GM-CSF protocols showing slightly better performance. Notably, combining protocols with tumor conditioned medium led to MDSCs with decreased immunosuppressive capabilities. These findings contribute to our understanding of the optimal conditions for the tuning of specific characteristics of MDSCs during generation *ex vivo*. The results may facilitate the development of novel approaches to correct the immunosuppressive properties of MDSCs and enhance the targeting of therapeutics to these cells.

References

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