DOI:

FENOTIPSKE I FUNKCIONALNE KARAKTERISTIKE HUMANIH MIJELOIDNIH SUPRESORSKIH ĆELIJA DIFERENTOVANIH IN VITRO OD MONOCITA POD UTICAJEM FAKTORA STIMULACIJE GRANULOCITNIH/MAKROFAGNIH KOLONIJA, INTERLEUKINA 6 I PROSTAGLANDINA E2

PHENOTYPIC AND FUNCTIONAL CHARACTERISTICS OF HUMAN MYELOID SUPPRESSOR CELLS DIFFERENTIATED IN VITRO FROM MONOCYTES UNDER THE INFLUENCE OF GRANULOCYTE/MACROPHAGE COLONY STIMULATING FACTOR, INTERLEUKIN 6 AND PROSTAGLANDIN E2

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SAŽETAK

Uvod: Mijeloidne supresorske ćelije (MDSC), otkrivene kao jedan od glavnih faktora koji pokreću progresiju tumora, takođe su uključene u povoljne efekte u autoimunskim bolestima, ali njihova uloga nije u potpunosti poznata.
Ciljevi: Protokoli za diferencijaciju MDSC dobijenih iz humanih monocita in vitro nisu u potpunosti uspostavljeni, posebno sa aspekta uloge prostaglandina E2 (PGE2) u indukciji MDSC in vivo, stoga je cilj našeg istraživanja bio da vidimo koji je protokol najbolji za dieferencijaciju MDSC.
Metodologija: Metodologija istraživanja obuhvatala je izolaciju mononuklearnih ćelija iz periferne krvi korišćenjem metoda adherencije i magnetnog sortiranja. Te ćelije su dalje diferencirane u monocitne mijeloidne supresorske ćelije (M-MDSC) i nezrele dendritske ćelije (iDC), a zatim su analizirane njihove morfološke i funkcionalne karakteristike, uključujući supresivnu aktivnost i sposobnost indukcije polarizacije T ćelija. Za procenu fenotipskih i funkcionalnih promena korišćene su tehnike kao što su protočna citometrija, analiza citokina i statistička obrada podataka.
Rezultati: Naši rezultati su pokazali da su GM-CSF i IL-6 neophodni za indukciju CD33+HLA- DRlowCD14+CD209-MDSC in vitro.. Međutim, dodavanje PGE2 koktelu GM-CSF/IL-6 dodatno je povećao ekspresiju CD14 i CCR7 a smanjio ekspresiju CD1a, HLA-DR, CD209, CD16, CD11c, CD11b na ovim ćelijama. MDSC indukovane u prisustvu PGE2 ispoljile su više nivoe CD39, PD1L, ILT-3 i ILT-4, i produkovale su veće koncentracije TGF-ß i IL-27, i manje koncentracije IL-1ß, IL-10, IL-23 i TNF-α nakon stimulacije LPS/IFN-γ, u poređenju sa MDSC indukovanim sa GM-CSF/IL-6. U populaciji aloreaktivnih T ćelija koje su kultivisane zajedno sa PGE2 indukovanim MDSC bilo je više CD4+GATA-3+ T ćelija koje produkuju IL-4 a manje CD4+RORγt+ T ćelija koje produkuju IL-17. Takođe bila je veća zastupljenost CD8+ T ćelija koje produkuju IFN-γ ali ne i CD4+IF N-γ+ T ćelija. Interesantan je nalaz je da su PGE2- MDSC smanjivale procenat aloreaktivnih CD4+CD25hiFoxP3+TGF-ß Tregs, ali su značajno povećale procenat CD4+IL-10+FoxP3-IL-4- Tr-1 i CD8+IL10+ T ćelija, u poređenju sa MDSC indukovanih samo u prisustvu GM-CSF/IL-6.
Zaključci: U zaključku se može istaći da PGE2 potencira supresivni fenotip i funkcije MDSC indukovanih u prisustvu GM-CSF/IL-6 i da je ovaj protokol za diferencijaciju MDSC pokazao najbolje rezultate, naročito jer mijenja mehanizme uključene u indukciju Tregs, što bi moglo biti važno od značaja u razvoju strategija fokusiranih na terapijske efekte MDSC kod tumora i autoimunskih bolesti.
Ključne reči: MDSC, prostaglandin E2, Interleukin 6

ABSTRACT

Introduction: Myeloid derived suppressor cells (MDSC), discovered as one of the major factors driving tumor progression, are also involved in beneficial effects during the course of autoimmune diseases, but their role is not fully understood.
Aim: The protocols for the in vitro differentiation of MDSCs derived from human monocytes have not been fully established, particularly regarding the role of prostaglandin E2 (PGE2) in the induction of MDSCs in vivo. Therefore, the aim of our research was to determine which protocol is most effective for MDSC differentiation.
Methods: The methodology involved isolating mononuclear cells from peripheral blood using adherence and magnetic sorting techniques. These cells were then differentiated into monocytic myeloid-derived suppressor cells (M-MDSC) and immature dendritic cells (iDC), with subsequent analysis of their morphology and functional characteristics, including suppressive activity and ability to induce T cell polarization. Techniques such as flow cytometry, cytokine analysis, and statistical evaluation were used to assess phenotypic and functional changes under the influence of various factors like GM-CSF, IL-6, PGE2, LPS, and IFN-γ
Results: Here we found that GM-CSF and IL-6 were required for the induction of CD33+HLA-DRlowCD14+CD209- MDSC in. However, the addition of PG-E2 to GM-CSF/IL-6 cocktail up-regulated additionally CD14 and CCR7, and down-regulated the expression of CD1a, HLA-DR, CD209, CD16, CD11c, CD11b on these cells. PG-E2-induced MDSC expressed higher levels of CD39, PD1L, ILT-3 and ILT-4, and produced higher amounts of TGF-ß and IL-27, and lower amounts of IL- 1ß, IL-10, IL-23 and TNF-α after LPS/IFN-γ stimulation, compared to GM-CSF/ IL-6-induced MDSC. Alloreactive T cells co-cultured with PG-E2-induced MDSC contained more IL-4- producing CD4+GATA-3+ T cells, and less IL-17-producing CD4+ RORγt+ T cells, as well as IFN-γ-producing CD8+ T cells, but not CD4+IFN-γ+ T cells. Interestingly, PG-E2-induced MDSC generated a smaller percentage of alloreactive CD4+CD25hiFoxP3+TGF-ß Tregs, but increased significantly the percentage of CD4+IL-10+FoxP3-IL-4- Tr-1 and CD8+IL10+ T cells, compared to GM-CSF/IL-6-induced MDSC.
Conclusion: Therefore, PG-E2 potentiate the suppressive phenotype and functions of GM-CSF/IL-6-induced MDSC, and that this protocol for MDSC differentiation showed the best results, especially it changed the mechanisms involved in Treg induction, which could be important in development of therapeutic strategies focused on MDSC-related effects in tumors and autoimmune diseases.
Key words: MDSC, prostaglandin E2, interleukin 6