https://hdl.handle.net/20.500.12512/239542

Allogenic mesenchymal stromal cells and their extracellular vesicles modulate inflammation via cytotoxic and regulatory T lymphocyte populations. A pilot in-vitro study

Valiukevičius, Paulius; Bagdonas, Dovydas; Mačiulaitis, Justinas; Insodaitė, Rūta; Čiapienė, Ieva; Gasiūnienė, Edita; Gradauskienė, Brigita; Mačiulaitis, Romaldas

Introduction: Mesenchymal stromal cells (MSC) and their extracellular vesicles (EV) have immunomodulatory properties (1,2), which are therapeutic in a wide spectrum of diseases (3). Potency assays are crucial to ensure consistency and quality, as well as to optimize dosage (4). One possible approach is the peripheral blood mononuclear cell (PBMC) co-culture assay. MSC and their exosomes can suppress stimulated PBMC proliferation; however, there is a lack of understanding of which populations of PBMC are affected the most. In this study, we focus on evaluating the effect of MSC or their EV on the proliferation of T regulatory and CD8+ lymphocytes, which are important in the pathogenesis of many inflammatory and autoimmune diseases (5,6). Methods: A placenta was collected from a healthy volunteer who underwent a cesarean section after obtaining written informed consent. Venous blood samples were collected from three healthy volunteers with written informed consent. All procedures were performed in accordance with Lithuanian Bioethics Committee standards (approval no. BE-2-105). MSC were isolated from the placenta by collagenase digestion. Cells were cultured in MEM supplemented with 10% FBS and 0.1% gentamicin. Fourth passage cells were used in the experiments. The conditioned medium was collected, filtered through a 0.22 µm filter, and concentrated with a tangential flow filtration 500 kDa filter 30 times to concentrate the EV. PBMC co-culture was performed as described by Oliver-Vila et al. (7). One million cells from each donor were frozen for use as a baseline for the gene expression assay. PBMC (5×10^5 per well) were cultured with either 2×10^5 MSC, 12 µL of EV, or vehicle (control) in duplicate for five days. PBMC were collected, washed, and incubated with monoclonal anti-human antibodies CD3-eFluor450, CD4-Pacific Orange, CD8-APCCyanine7, CD25-APC, CD127-PE-Cyanine7, and viability dye 7-AAD (Invitrogen). At least 100 000 cells were acquired using a BD FACSLyric flow cytometer and analyzed with FlowJo. Fluorescence minus one (FMO) and isotype controls were used. The data were analyzed using FlowJo. PBMC gene expression for ACTB (reference gene) and IL-10 was analyzed using TaqMan® Gene Expression Assays (Applied Biosystems). Gene expression levels were calculated using the ΔΔCt method (8). Results are described as medians with the 25–75th interquartile range (IQR). Statistical analysis was performed using GraphPad Prism, with a significance level of $p<0.05$. Differences across groups were evaluated using the Kruskal-Wallis test, and Dunn's test was used for pairwise comparisons. Results: Stimulation of PBMCs with PMA and ionomycin resulted in robust proliferation. Additionally, it increased the variability in lymphocyte size and granularity and modulated the expression of surface markers, making it difficult to separate the positive and negative populations. Therefore, FMO controls were used to select a cut-off value for negative populations. Viable CD3+ cells (lymphocytes) were selected and further gated to CD4+CD8- (T helpers) and CD4-CD8+ (cytotoxic T lymphocytes) populations. T regulatory lymphocytes (Treg) were defined from the CD4+CD8- population by gating on cells with high expression of CD25 and low or negative expression of CD127 (CD4+CD25highCD127low/-) (9). The normalized proliferation of CD3+, CD4-CD8+, and Treg populations was analyzed in all groups. The proliferation rates in the control sample were normalized to a baseline value of 1 for accurate comparative analysis. Furthermore, the frequency of CD4-CD8+ in all CD3+ cells and Treg cells in CD4+CD8− cells was analyzed in all groups. CD3+ normalized proliferation in MSC samples was 0.75 (0.65-0.80), in EV samples - 0.64 (0.55- 0.79), with a reduction in both treatment groups ($p<0.05$). CD4-CD8+ proliferation was also significantly reduced in both groups 0.46 (0.46-0.50) for MSC and 0.88 (0.87-0.93) for EV ($p<0.05$). Interestingly, only MSC statistically significantly ($p<0.01$) reduced the proliferation of Treg, resulting in a normalized proliferation of 0.12 (0.08-0.41). There was a trend of lower CD4-CD8+ frequency in the MSC group (15.0 (4.9-22.0) % control vs. 6.2 (4.8-13) % MSC), which did not reach statistical significance. Interestingly, there was also a trend of increased Treg frequency in the treatment groups (control 15.0 (9.0-20.0) %, MSC 19.0 (13.0- 36.0) % and EV 18.0 (7.9-23.0) %), which did not reach statistical significance. IL-10 gene expression fold changes from baseline in control, MSC, and EV groups were 0.47 (0.35-0.50), 2.8 (2.8-6), and 0.91 (0.4-1.5), respectively, with a significant elevation only in the MSC group ($p<0.01$). Conclusions: Both MSC and EV in selected doses reduced the proliferation of lymphocytes and their CD4-CD8+ population, but only MSC reduced the proliferation of Treg lymphocytes. Furthermore, MSC co-culture resulted in significant upregulation of IL-10, which is the main effector molecule of T regulatory lymphocytes (10). This suggests that MSC therapy may modulate Treg numbers not through increased proliferation but possibly by altering the relative proportions of both Treg and cytotoxic lymphocytes within the overall lymphocyte population and thus shifting the balance towards an anti-inflammatory immune response.

Detali informacija

Publikacijos rūšis	Tezės kitame recenzuojamame leidinyje / Theses in other peer-reviewed publication (T1e)

Antraštė	Allogenic mesenchymal stromal cells and their extracellular vesicles modulate inflammation via cytotoxic and regulatory T lymphocyte populations. A pilot in-vitro study
Leidėjas	2023
Publikuota	2023-10-19
Leidinyje	International Seminar "Clinical Application of Stem Cells in Kidney Transplantation and Nephrology" : 19 October 2023, Kaunas, Lithuania : Abstract Book / Lithuanian University of Health Sciences. Hospital of Lithuanian University of Health Sciences Kauno Klinikos. Lietuvos Nefrologijos, Dializės ir Transplantacijos Asociacija.
ISBN (leidinio)	978-9955-15-806-6
Puslapis nuo	10
Puslapis iki	11
Mokslo kryptis	Medicina / Medicine (M001)
	Biologija / Biology (N010)
OECD klasifikacija	Medical and Health sciences::Basic medicine::Immunology
	Medical and Health sciences::Clinical medicine::Urology and nephrology
Autorius	Valiukevičius, Paulius [MA Medicinos fakultetas (U520000), LT]
	Bagdonas, Dovydas [Imunologijos ir alergologijos klinika (U526600), LT]
	Mačiulaitis, Justinas [Fiziologijos ir farmakologijos tyrimų laboratorija (U520410), LT] [Kardialinės patologijos laboratorija (U590300), LT]
	Insodaitė, Rūta [Fiziologijos ir farmakologijos institutas (U520400), LT]
	Čiapienė, Ieva [Molekulinės kardiologijos laboratorija (U590800), LT]
	Gasiūnienė, Edita [Imunologijos laboratorija (U526610), LT]
	Gradauskienė, Brigita [Imunologijos ir alergologijos klinika (U526600), LT]
	Mačiulaitis, Romaldas [Fiziologijos ir farmakologijos institutas (U520400), LT]
Meno kūrinio tipas	Originalus / Original
Mokslo kryptis	Medicina / Medicine (M001)
	Biologija / Biology (N010)
Documento rūšis	Pranešimas konferencijoje / Conference Paper
Šalis	Lietuva / Lithuania (LT)
Kalba	Anglų / English (en)
URI	https://hdl.handle.net/20.500.12512/239542

Bibliografinė informacija	10
Prieskyra	Kardialinės patologijos laboratorija (U590300)
	Medicinos Akademija
	MA Kardiologijos institutas
	MA Medicinos fakultetas
	Lietuvos sveikatos mokslų universitetas
	Fiziologijos ir farmakologijos tyrimų laboratorija (U520410)
	Molekulinės kardiologijos laboratorija (U590800)
	Imunologijos laboratorija (U526610)
	Imunologijos ir alergologijos klinika
	Fiziologijos ir farmakologijos institutas
Peržiūros	7

Finansavimas

Pavadinimas
Research Council of Lithuania Research Council of Lithuania