Cord blood MDSCs polarize T-cells towards a TH2-response and modulate phenotype of monocytes

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Background:

Infection is the leading cause of neonatal morbidity and mortality. Newborns show an impaired anti-microbial host defense, but the underlying mechanisms are not fully understood. Neonatal T-cell immunity is characterized by a polarization towards an anti-inflammatory TH2-cytokine response and a diminished TH1-response. Furthermore T cell activation is hampered by diminished capacity for antigen presentation and costimulation by antigen presenting cells, such as neonatal monocytes, which are characterized by low HLA-DR-expression and low activation of CD80. The reasons for this phenotype are unclear. Myeloid derived suppressor cells (MDSCs) are myeloid progenitor cells with suppressive capacities on other immune cells and have been shown to accumulate in human cord blood. In the present study we analyzed the effects of cord blood MDSCs (CB-MDSCs) on the phenotype of CD4 T-cells and monocytes.

Methods:

- Quantification of CD66b+/CD33+/CD14-/HLA-DRlow/- MDSCs in cord blood and peripheral blood of children of different ages
- Enrichment of CB-MDSCs by magnetic activated cell sorting
- Five days Co-culture of PBMCs alone or PBMCs with CB-MDSCs
- Surface staining for CD4, CCR4, CCR6, CXCR3, CD14, HLA-DR, CD80 and CD86 and intracellular staining for IL-4 and IFN-γ

Results:

Figure 1: MDSCs accumulate in cord blood

MDSCs were quantified in cord blood (CB) from healthy newborns and peripheral blood from children of different ages. Scatter diagram shows percentages of MDSCs in CB and peripheral blood. n=5-58, p=0,0003, Kruskal-Wallis-Test und Dunn´s multiple comparison test for non-parametric data.

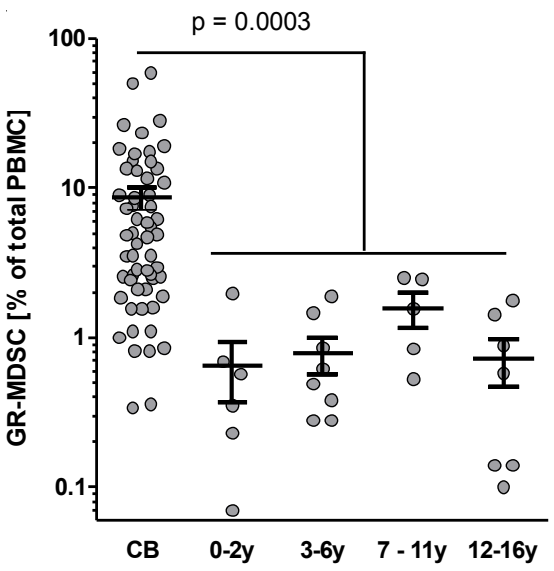
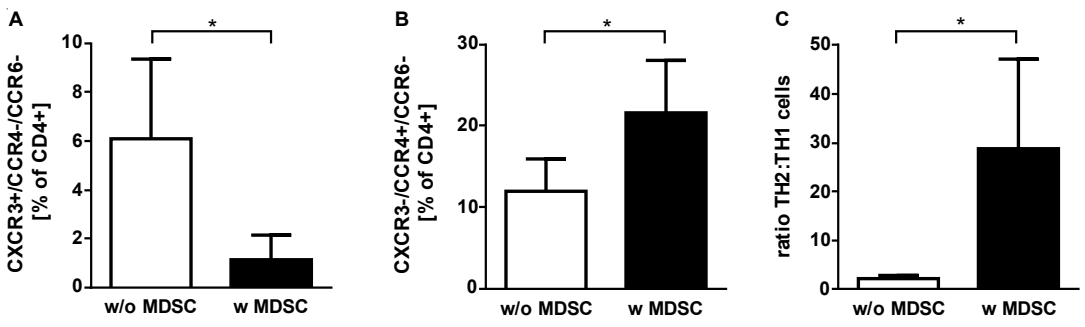
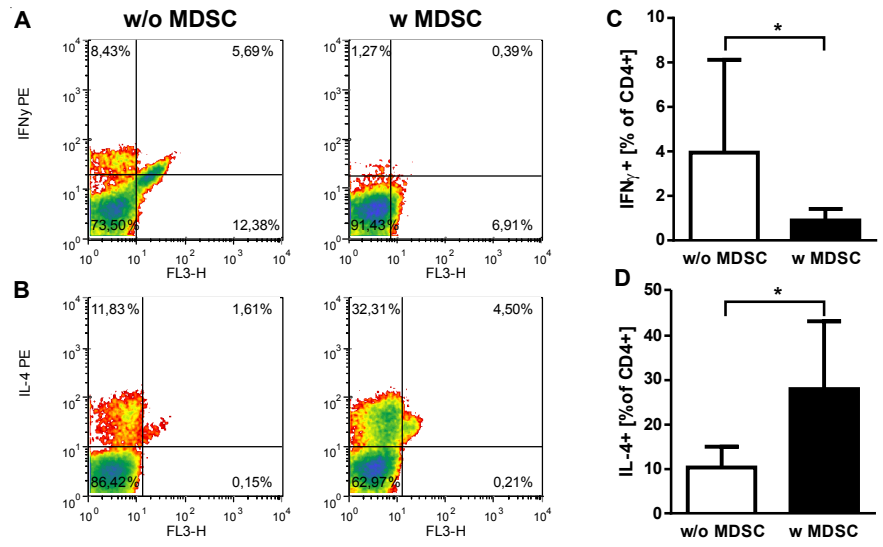


Figure 2: CB-MDSCs polarize T-cells towards a TH2 phenotype



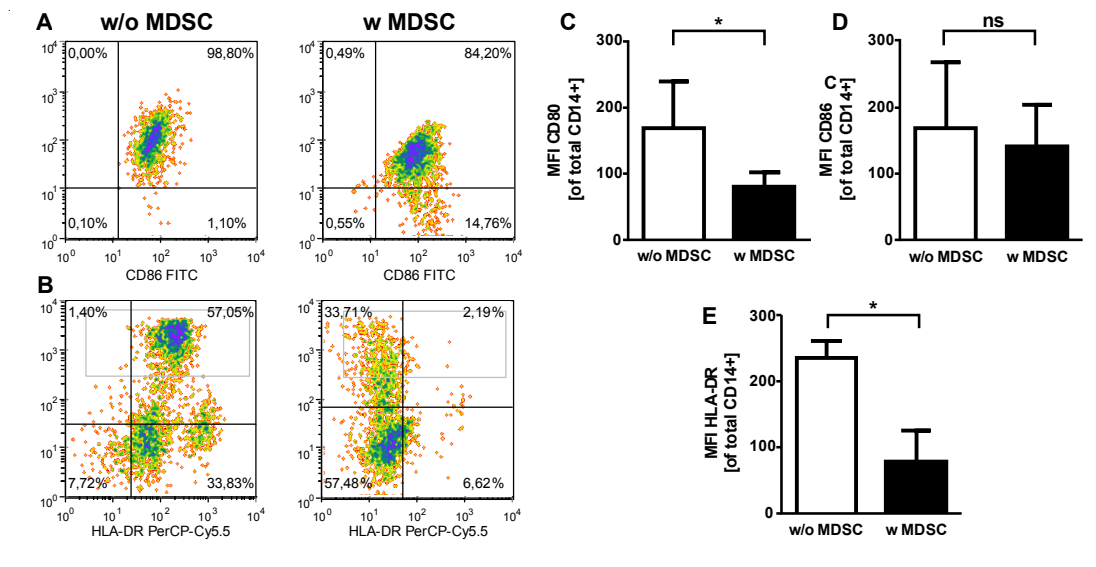
CB-MDSCs were enriched from CBMCs and added to PBMCs isolated from a healthy adult control. After five days of culture percentage of CD4+/CXCR3+/CCR4-/CCR6- TH1 cells and CD4+/CXCR3-/CCR4+/CCR6- TH2 cells were determined by flow cytometry. (A, B) Bar graphs show the percentage of CXCR3+/CCR4-/CCR6- cells and CXCR3-/CCR4+/CCR6- cells of CD4+ cells without addition of CB-MDSCs (white bars) and with addition of CB-MDSCs (black bars). (C) Ratio of TH2- to TH1-cells without (white bars) and with addition of CB-MDSCs (black bars). Bars represent pooled data from 6 independent experiments and mean and standard deviation is indicated. *p <0.05; Wilcoxon matched-pairs signed rank test.

Figure 3: CB-MDSCs polarize T-cells towards a TH2 cytokine response



CB-MDSCs were enriched from CBMCs and added to PBMCs isolated from a healthy adult control. After five days of culture cells were stimulated for 5 hours with Golgiplug and intracellular staining for IFNγ and IL-4 was performed. (A, B) density plots for FL-1 versus IFNγ (A) and FL-1 versus IL-4 (B) without addition of CB-MDSCs (w/o MDSC) and with addition of CB-MDSCs. (C, D) Bar graphs show the percentage of IFNγ+cells and IL-4+cells of CD4+ cells without addition of CB-MDSCs (white bars) and with addition of GR-MDSCs (black bars). Bars represent pooled data from 6 independent experiments and mean and standard deviation is indicated. *p <0,05; Wilcoxon matched-pairs signed rank test.

Figure 4: CB-MDSC induce downregulation of HLA-DR and constimulatory molecule CD80 on monocytes



CB-MDSCs were enriched from CBMCs and added to PBMCs isolated from a healthy adult control. After five days of culture cells were stained for CD14, HLA-DR, CD80 and CD86. (A, B) density plots for CD86 versus CD80 (pregated on CD14) (A) and HLA-DR versus CD14 (B) without addition of CB-MDSCs (w/o MDSC) and with addition of CB-MDSCs. (C, D, E) Bar graphs show the mean fluorescence intensity (MFI) of CD80 (A), CD86 (B) and HLA-DR (C) on CD14+ monocytes without addition of CB-MDSCs (white bars) and with addition of GR-MDSCs (black bars). Bars represent pooled data from 6 independent experiments and mean and standard deviation is indicated. *p <0,05; Wilcoxon matched-pairs signed rank test

Conclusion:

MDSCs accumulate in cord blood, polarize T-cells towards a TH2 response and lead to a downregulation of HLA-DR and costimulatory molecule CD80 on monocytes. These results point again towards a role of MDSCs for regulating neonatal immune responses. Modulation of MDSC function could be a potential therapeutic target in neonatal infections.

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