Stem Cell Reports, Volume *9*

Supplemental Information

Activated Tissue-Resident Mesenchymal Stromal Cells Regulate Natural Killer Cell Immune and Tissue-Regenerative Function

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Supplemental Information

Human bone marrow MSC isolation and culture

Mononuclear cells from bone marrow aspirates were isolated by density gradient centrifugation using Leucosep tubes (Greiner Bio-One, Frickenhausen, Germany) according to the manufacturer's protocol and with approval by the Ethics Committee of the University Hospital of Essen. After washing with Dulbecco's Phosphate Buffer Saline (DPBS; Gibco Life Technologies, Darmstadt, Germany) the cell pellet was resuspended in "MSC-medium" composed of Dulbecco's modified Eagle's medium (DMEM; Gibco Life Technologies) supplemented with 10 % endotoxin-tested fetal calf serum (FCS; Biochrome, Berlin, Germany), 100 U/ml penicillin, 0.1 mg/ml streptomycin, 2 mM l-glutamax, and 1 mM sodium pyruvate (all from Sigma, Deisenhofen, Germany). One million mononuclear cells were seeded in 7 ml MSC-medium per T75 cm^2flask (Greiner Bio-One) and were incubated in a humidified atmosphere containing 5 % CO_2 at 37 °C. After three passages, phenotypical characterization was performed by flow cytometry. All MSCs were negative for the hematopoietic or endothelial markers CD45, CD34, and CD31 but expressed the characteristic MSC markers CD29, CD73, CD90, and CD105. MSCs were used in passages 3–7 throughout the experiments.

SUPPLEMENTARY FIGURE 1
Bone Marrow MSC regulation of NK

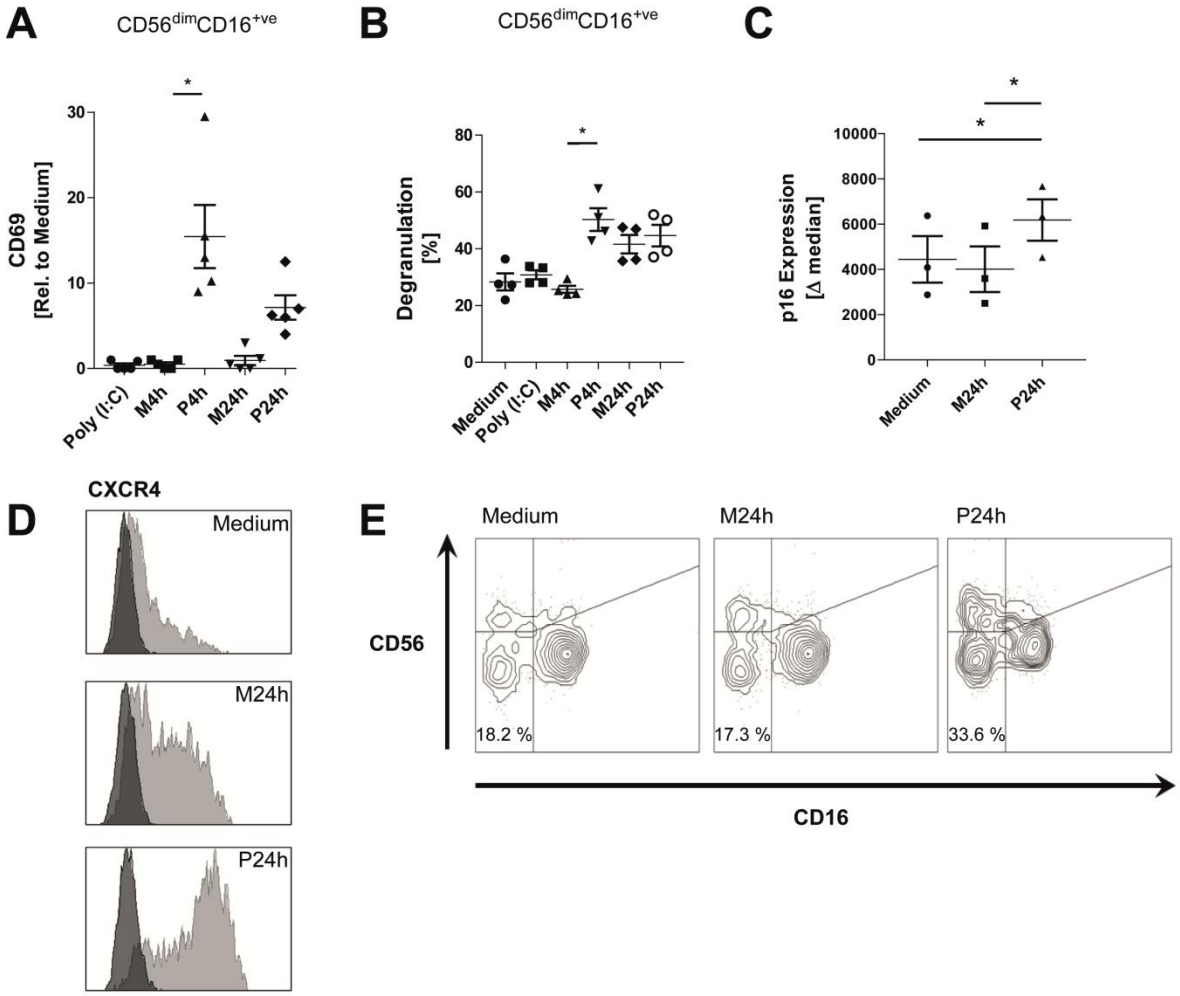

Figure legend for supplementary figure 1.

Supplementary Figure 1: Modulation of NK by poly(I:C)-stimulated bone-marrow (bm) MSC. (A) NK were incubated overnight (18 h) with SN from poly(I:C)-stimulated bmMSC (P4h and P24h), unstimulated bmMSC SN (M4h and M24h), poly(I:C) alone and medium. The expression of NK phenotypic marker CD69 on the CD56dimCD16+ve NK subset was measured. The delta median (marker expression minus the isotype) is shown (n = 4). (B) NK were incubated overnight with the indicated conditioned media derived from bmMSC. Degranulation of CD56dimCD16+ve NK cells against K562 was assessed using the CD107a assay (n = 4). (C) NK were treated for 1 day with

conditioned media derived from bmMSC and the expression of CDKN2A/p16INK4a (P16) was determined by flow cytometry (n = 3). (D & E) NK were incubated for 5 days in conditioned media derived from bmMSC, NK were then stained for CXCR4, CD56 and CD16 (CXCR4 is shown as histograms of whole NK population and scatter plots show NK subset distribution and % of CD56dim/CD16-ve NK, one representative experiment of 5). Data analysis was performed using a paired two tailed t-test, a p-value of <0.05* was considered significant.

Supplementary table 1.

Gene	***Sequence***	***Annealing temperature (C^o)***
B-actin	Forward AGCGGGAAATCGTGCGTG Reverse GGGTACATGGTGGTGCCG	60
COX-2	Forward TTCAAATGAGATTGTGGAAAAAT Reverse AGATCATCTCTGCCTGAGTATCTT	50
IDO	Forward TGTGAACCCAAAAGCATTTTTC Reverse AAAGACGCTGCTTTGGCC	60
SDF-1a	Forward TGAGCTACAGATGCCCATGC Reverse TTCTCCAGGTACTCCTGAATCC	62
SMA	Forward TGGCTATTCCTTCGTTACTA Reverse CGATCCAGACAGAGTATTTGC	58
SOCS1	Forward CTCGCATCCCCCTCAACC Reverse CATCCGCTCCCTCCAACC	60

SOCS3	Forward AGAAGATCCCCCTGGTGTTGA Reverse GCCCTTTGCGCCCTTTAC	60
uPAR	Forward CCACTCAGAGAAGACCAACAGG Reverse GTAACGGCTTCGGGAATAGGTG	60
VEGF	Forward CAAGATCCGCAGACGTGTAA Reverse TCTGTCGATGGTGATGGTGT	60