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Mesenchymal Stromal Cells Reprogram Neutrophils into Immunosuppressive Effectors via Phagocytosis and Extracellular Vesicle Transfer

Luana Tomaipitinca
Cellcolabs AB
Katia Lefsihane
Cellcolabs AB
André Görgens
Karolinska Institutet
Lina Sörvik
Cellcolabs AB
Jan Fredrik Talts
Cellcolabs AB
Petter Höglund
Karolinska Institutet
Katarina Le Blanc
Karolinska Institutet
Roland Fiskesund
Karolinska Institutet
Nadir Kadri
nadir.kadri@ki.se

Karolinska Institutet

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Abstract

Background

Mesenchymal stromal cells (MSCs) possess potent immunomodulatory properties, but the mechanisms through which they shape innate-adaptive immune interactions remain poorly understood. Given the functional plasticity of neutrophils, we investigated whether MSCs induce an immunoregulatory neutrophil phenotype capable of suppressing autologous T-cell responses and examined the mechanisms involved.

Methods

Ex vivo models were used to investigate MSC-neutrophil interactions and the capacity of MSC-conditioned neutrophils to modulate activated autologous T-cells. Direct and transwell co-culture systems were employed to evaluate the roles of cell contact and soluble mediators.

Results

Neutrophils acquired MSC-derived material through an oxygen-dependent phagocytosis process, which induced a regulatory phenotype referred to as “programmed neutrophils”. Compared with untreated controls, these cells exhibited enhanced survival and effectively suppressed autologous T-cell responses by limiting early activation events, as evidenced by reduced CD25 expression prior to HLA-DR upregulation.

T-cell suppression required direct neutrophil-T-cell interaction, whereas neutrophil programming occurred independently of physical contact with MSCs. MSC-conditioned medium alone was sufficient to influence neutrophil phenotype, implicating MSC-derived extracellular vesicles (EVs) as mediators of the reprogramming signal. Furthermore, both viable and apoptotic MSCs generated comparable effects, supporting a central role for MSC-driven neutrophil reprogramming in the development of immunosuppressive neutrophils.

Conclusions

Our study uncovers a novel MSC-neutrophil immunoregulatory pathway in which MSC-programmed neutrophils acquire suppressive activity and regulate adaptive immune responses. This mechanism enables MSCs to indirectly control T-cell activation and highlights neutrophil reprogramming as a central component of MSC immunomodulation, with potential applications in the development of potency assays and biomarkers for MSC-based therapies.

INTRODUCTION

The clinical relevance of mesenchymal stromal cell (MSC)–mediated immunosuppression has been most convincingly demonstrated in the context of acute graft-versus-host disease (GvHD), a life-

threatening complication of allogeneic hematopoietic stem cell transplantation driven primarily by excessive donor T-cell activation and tissue infiltration [1]. In December 2024, the U.S. Food and Drug Administration approved remestemcel-L-rknd (Ryoncil), an allogeneic bone marrow–derived MSC product, for the treatment of pediatric steroid-refractory acute GvHD, marking the first FDA approval of an MSC-based therapy in the United States and validating decades of translational research in this field, as highlighted by the International Society for Cell & Gene Therapy (ISCT) MSC Committee [2, 3]. This landmark approval reinforces the central importance of T-cell suppression as a clinically meaningful MSC function and underscores the value of robust *in vitro* systems that interrogate T-cell activation from multiple mechanistic angles.

Although adaptive immune pathology has traditionally been framed as a T-cell-driven process, it is increasingly clear that innate immune cells actively shape disease initiation, amplification, and tissue pathology. Among these, neutrophils emerge as early and abundant contributors at inflamed sites, where they promote epithelial and tissue damage, cytokine amplification, and enhanced antigen presentation that collectively fuel pathological T-cell activation [4, 5, 6, 7, 8]. Importantly, neutrophils are no longer viewed solely as short-lived pro-inflammatory effectors; rather, they are now recognized as highly plastic cells capable of adopting immunoregulatory phenotypes in response to contextual cues [9, 10, 11]. Despite this paradigm shift, whether MSCs therapeutically exploit neutrophils as secondary immunoregulatory effectors to restrain pathological T-cell activation remains largely unexplored.

Controlled *in vitro* T-cell suppression assays therefore provide a powerful and reductionist framework to dissect how MSCs modulate immune networks beyond direct MSC-T-cell interactions. Defining whether MSCs can reprogram neutrophils to suppress T-cell activation, and through which cellular and molecular mechanisms, may yield critical insight into MSCs mode of action in various diseases and potentially inform the rational design of next-generation MSCs or MSCs-derived therapies with enhanced potency, durability, and predictability.

At a mechanistic level, MSCs-mediated immune regulation is increasingly understood to rely on a combination of direct cell-cell interactions and extracellular vesicle (EV)-mediated communication, which together orchestrate context-dependent immune outcomes [12, 13, 14]. In T-cells, MSCs suppress activation, proliferation, and effector differentiation through contact-dependent inhibitory pathways as well as through EV-mediated transfer of immunoregulatory proteins, lipids, and nucleic acids [15, 16, 17]. Parallel work in macrophages has demonstrated that MSCs can induce stable anti-inflammatory or regulatory states via phagocytic uptake, metabolic rewiring, and EV-driven signaling, thereby reshaping downstream adaptive immune responses [18, 19, 20, 21]. These findings support a broader model in which MSCs act as transient instructors of host immunity rather than as long-lived effector cells.

In striking contrast, the mechanisms by which MSCs interact with neutrophils remain poorly defined. Although neutrophils are professional phagocytes and abundant recipients of EVs in vivo [22, 23], their capacity to integrate MSC-derived signals and translate them into durable, T-cell-modulating functions has not been systematically addressed. In particular, whether neutrophil programming requires direct

physical interaction with MSCs, phagocytic uptake of MSC material, EV-mediated cargo transfer, or a coordinated combination of these mechanisms, remains unknown. Even less is understood about how such programmed neutrophils subsequently regulate the magnitude, timing, and persistence of T-cell suppression.

Mechanistic insights from broader immunology literature indicate that neutrophil regulation of T-cell immunity is highly structured and spatially constrained [24]. Neutrophils can engage activated T-cells through adhesion-dependent, immunological-synapse–like contacts, enabling delivery of short-range inhibitory signals and creation of metabolically restrictive microenvironments [25]. Contact-dependent suppressive programs have been described, including integrin-mediated interactions and reactive oxygen species–linked inhibitory mechanisms that require close cellular proximity. In parallel, accumulating evidence supports a more direct immunoregulatory role for neutrophils via antigen-presentation-adjacent functions, with human neutrophils shown to express molecular components that facilitate T-cell engagement and, in certain contexts, modulate T-cell proliferation in an adhesion-dependent manner [26, 27, 28, 29]. Collectively, these observations establish neutrophils as tunable regulators of adaptive immunity, with outcomes ranging from immune activation to suppression depending on activation state, tissue context, and the molecular architecture of cell-cell contact.

Beyond direct contact, neutrophils also participate in EV-based immune communication. Neutrophil-derived EVs, including exosomes and ectosomes, carry bioactive cargo capable of shaping inflammatory set-points and immune effector functions [30]. Importantly, these vesicles can exert either pro- or anti-inflammatory effects depending on the stimulus and vesicle composition, supporting the view that EV release is an active and regulated arm of neutrophil biology rather than passive cellular debris. EV-mediated pathways therefore represent a plausible mechanism through which neutrophils may propagate immunoregulatory programs downstream of instructive cues, consistent with observations across disease contexts in which EV exposure imprints suppressive phenotypes, including inhibition of T-cell responses [31, 32, 33].

Here, we address these critical gaps by defining how MSCs program neutrophils into immunosuppressive effectors capable of restraining T-cell activation. By integrating functional T-cell suppression assays with mechanistic dissection of contact dependence, phagocytosis, oxygen sensitivity, and EV-mediated signaling, our study establishes neutrophils as key intermediaries of MSC-driven immune regulation. These findings provide a unifying framework linking MSC clearance, innate immune reprogramming, and adaptive immune suppression, with direct relevance to GvHD and other inflammatory diseases treated with MSC-based therapies.

METHODS

Blood processing and neutrophil and T-cell isolation

Neutrophils were isolated as previously described [34]. Briefly, whole heparin blood was diluted 1:1 in PBS-10% heparin-plasma and stratified on Ficoll (Merck, Darmstadt, Germany; cat. no. GE17-1440-02) for gradient separation. Mononuclear cells were collected to perform T-cell isolation using the human Pan T-cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany; cat. no.130-096-535). The granulocyte layer was collected and mixed with Dextran (Merck, Darmstadt, Germany; cat. no. 31392) to a final concentration of 0.8% Dextran. Following Dextran-based separation of red cells, the leukocyte enriched cell suspension was subjected to magnetic separation with EasySep™ Direct Human Neutrophil Isolation Kit (STEMCELL Technologies, Vancouver, BC, Canada; cat. no. #19666). Neutrophils were then resuspended at the concentration of 10^6/ml in RPMI-1640 (Gibco, Thermo Fisher Scientific, Waltham, MA, USA; cat. no. 61870-010) supplemented with 10% FBS (Gibco, Thermo Fisher Scientific, Waltham, MA, USA; cat. no. A5670801).

For experiments where whole heparin blood was co-cultured with MSCs, blood leukocytes were counted. Calculating that granulocytes represent 60–70% of leukocytes in blood, the blood was then resuspended in RPMI-10%FBS to bring the granulocyte concentration to 10^6/ml.

MSCs isolation and preparation for co-culture

Passage 2 GMP-grade manufactured human bone marrow MSCs were provided by Cellcolabs AB. MSCs were then expanded up to passage 3 in Dulbecco´s Modified Eagle Medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA; cat. no. 31885023) supplemented with 5% Human Platelet Lysate (PLBioScience, Aachen, Germany; cat. No. PE21012) and cryopreserved in CryoStor 10 (Biolife Solutions, Washington, USA; cat. no. 210102) until use.

All donors underwent a comprehensive health assessment conducted by a primary care physician. This included a physical examination, an oral medical history assessment, review of electronic health records, a written self-reported health questionnaire, and blood analyses covering routine haematological and biochemical parameters. These assessments were performed to evaluate the donors' overall health and identify any potential disease-related risk factors. Prior to donation, donors were required to test negative on two separate occasions for HIV-1/2, HTLV-1/2, hepatitis B, hepatitis C, and syphilis. Donors were informed about the potential risks associated with the procedure and provided informed consent for bone marrow donation.

Bone marrow was obtained by aspiration from the posterior iliac crest under sterile conditions. For each production batch, MSCs were isolated and subsequently expanded from a single donor. Donor screening and bone marrow collection were conducted in compliance with applicable Swedish legislation and regulatory requirements, including SOSFS 2009:30, the regulations of the Swedish National Board of Health and Welfare concerning the donation and procurement of tissues and cells, and Act (2008:286) on quality and safety standards for the handling of human tissues and cells. The procedures were also conducted in accordance with Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004, establishing standards of quality and safety for the donation, procurement, testing,

processing, preservation, storage, and distribution of human tissues and cells.All MSCs used for co-culture experiments were thawed on the day of the experiments and resuspended in RPMI-10%FBS at the concentration of 10^6/ml.

To prepare late apoptotic MSCs, cells were submitted to heat shock by placing them in a water bath at the temperature of 52°C for 40min.

To prepare necrotic MSCs, cells were treated with 6M perchloric acid (Merck, Darmstadt, Germany; cat. no. 244252) for 6h.

Cell death status was assessed using the Violet Chromatin Condensation/Dead Cell Apoptosis Kit with Vybrant® DyeCycle™ Violet and SYTOX® AADvanced™ for Flow Cytometry (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA; cat. no. A35135), following manufacturer´s indications.

MSC and neutrophils co-culture experiments

Cell contact-based co-culture. All co-cultures between MSCs and neutrophils/whole blood were set up at 1:1 ratio for 2h, 6h or 18h (10^6 neutrophils and 10^6 MSCs, RPMI-10%FBS volume adjusted to reach a final cell concentration of 10^6/ml). Co-cultures were carried on in polypropylene 5ml FACS tubes (Falcon, Corning Incorporated, Corning, NY, USA; cat. no. 352063).

Cell cultures without cell-cell contact. 12 well 3µm-pore cell culture inserts were used (CellQART, SABEU GmbH & Co. KG, Northeim, Germany; cat. no. 9313002), (TPP, Techno Plastic Products AG, Trasadingen, Switzerland; cat. no. 92012). Neutrophils were seeded at the bottom of the well, while MSCs were seeded on the transwell inserts at 1:1 ratio ($0.35x10^6$ neutrophils and $0.35x10^6$ MSCs).

Preparation of conditioned and depleted media. Prior the experiments, neutrophils and MSCs or MSCs alone were cultured in polypropylene 5ml FACS tubes for 2h or 6h. The conditioned medium was then collected and centrifuged at 500g for 5min. The supernatant was then transferred into a new tube and centrifuged at 2000g for 10min to ensure removal of cells and debris. The supernatant was then stored at -20°C until further use. EVs were depleted from the conditioned medium by loading onto Amicon Ultra 0.5mL centrifugal filters (Ultracel 10K, Millipore, Darmstadt, Germany, cat. no. UFC501096) and spun 30min at 14.000 x g. Fluorescent reference EVs were used to test for successful depletion of EVs in the flow-through [35].

On the day of the experiment, naive neutrophils were resuspended at 10^6/ml in fresh RPMI-10%FBS mixed with 50% of either conditioned or depleted medium and incubated for 2h and 6h in polypropylene 5ml FACS tubes.

T-cell stimulation and suppression assay preparation

After co-incubation of neutrophils with MSCs, in cases where multiple co-incubations of the same sample were required to obtain sufficient cell numbers, the resulting cell suspensions were pooled; residual MSCs were then removed using human CD90 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany; cat. no. 130-096-253) and following manufacturer instructions for separation with LD columns (Miltenyi Biotec, Bergisch Gladbach, Germany; cat. no. 130-042-901). Neutrophils were then counted and resuspended in RPMI at 10^6/ml concentration.

Isolated T-cells were labelled with 5μM Cell Trace Violet (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA; cat. no. C34557) at 37°C for 20min, then washed in PBS (Gibco, Thermo Fisher Scientific, Waltham, MA, USA; cat. no. 14190-250) and resuspended 10^6/ml in RPMI-10%FBS. After staining, T-cells were activated with Dynabeads Human T-Activator CD3/CD28 beads (Gibco, Thermo Fisher Scientific, Waltham, MA, USA; cat. no. 11131D) and co-cultured with neutrophils at 1:1 ratio ($0.1x10^6$ neutrophils and $0.1x10^6$ T cells) in 96-well v-bottom plates (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA; cat. no. 277143) for 2, 4 and 7 days (final cell concentration: 10^6/ml).

For cell cultures without cell-cell contact, 12 well 3μm-pore transwells were used. T-cells were seeded at the bottom of the well, while neutrophils were seeded on the transwell inserts ($0.5x10^6$ neutrophils and $0.5x10^6$ T-cells).

Phagocytosis Assay

Right after thawing, MSCs were resuspended in PBS at 10^6/ml and labelled with 4μM pHrodo Red for 10min at RT (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA; cat. no. p36600). MSCs were then washed two times and resuspended in RPMI-10%FBS at 10^6/ml. Neutrophils/whole blood and MSCs were co-incubated at 1:1 ratio in RPMI-10%FBS at 10^6/ml for 2, 6 and 18h as described above. The phagocytosis rate was measured by pHrodo acquisition by neutrophils, using neutrophils treated with Cytochalasin D (Merck, Darmstadt, Germany; cat. no. C8273) as negative controls (50μg/ml Cytochalasin for whole blood and 10μg/ml Cytochalasin for purified neutrophils). The phagocytosis rate was also determined counting MSC number at the end of the co-culture by flow cytometry using Trucount beads (BD Biosciences, San Jose, CA, USA; cat. no. 663028).

Flow Cytometry Staining

The following antibodies were purchased from BD Biosciences (San Jose, CA, USA) and used for staining: AF647-CD66b (cat. no. 561645), BV410-CD14 (cat. no. 558121), BV510-CD16 (cat. no. 563830), PeCy7-CD90 (cat. no. 561558), PerCPCy5-CD66b (cat. no. 562254), APC-CD3 (cat. no. 555335), PE-CD25 (cat. no. 555432), PerCP-Cy5.5-HLA-DR (cat. no. 552764). Depending on the mix of antibodies, dead cells and debris were labelled with either LIVE/DEAD™ Fixable Green Dead Cell Stain Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA; cat. no. L34970) or eBioscience™ Fixable Viability Dye eFluor™ 780

(Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA; cat. no. 65-0865-14). Samples were then fixed using BD Phosflow™ Lyse/Fix Buffer 5X (BD Biosciences, San Jose, CA, USA; cat. no. 558049) and data were collected using either Cytoflex Flow Cytometer (Beckman Coulter, Brea, CA, USA) or the BD FACSymphony and analysed using FlowJo 10.8.1 software (BD Biosciences, San Jose, CA, USA).

EV Extraction and Analysis

The EV surface marker profiles and respective mean fluorescence intensities (MFI) for 37 different surface markers was assessed by multiplex bead-based EV flow cytometry (MACSPlex EV Kit IO, Miltenyi Biotec, Bergisch Gladbach, Germany; cat. no.130-108-813) as described before [36, 37]. Briefly, EV samples were diluted with MACSPlex buffer to a final volume of 60µL and incubated over-night with 10µL MACSPlex Exosome Capture Beads on an orbital shaker at room temperature (RT) in the dark. Beads were washed with MACSPlex buffer, and then 4µL of APC-conjugated CD9, CD63 and CD81 (Pan tetraspanin) detection antibodies were added to each sample, respectively, in a total volume of 135µL. Following incubation for 1h on an orbital shaker at RT, samples were washed again and incubated for another 15min before a final washing step was performed. Incubation and washing steps were performed in 0.22µm filter plates. Final samples were resuspended in 150µL MACSPlex buffer and acquired on a MACSQuant Analyzer 16 flow cytometer (Miltenyi Biotec). Data was analyzed with FlowJo software version 10.5.3 and expressed as log10-transformed fold change values over respective non-EV containing buffer controls as described before [38]. Heatmaps were generated with Morpheus (https://software.broadinstitute.org/morpheus).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA), and statistical significance was defined as $p < 0.05$. For experiments conducted in technical triplicate, the three replicate measurements were averaged and the resulting value was considered one observation for statistical analysis. In experiments designed to compare the effects of different MSC donors on neutrophils from the same donor, individual technical replicates are shown instead. NTPR analyses were performed at the biological donor level to avoid pseudoreplication. For comparisons involving more than two matched conditions, repeated-measures one-way, two-way ANOVA or mixed-effects model analysis were performed, followed by the appropriate post hoc multiple-comparisons test, as indicated in the corresponding figure legend. Comparisons between two matched conditions were performed using either a two-tailed paired t-test or a two-tailed Wilcoxon signed-rank test, as specified in the corresponding figure legends. Associations between variables were assessed using simple linear regression.

RESULTS

MSC-programmed neutrophils actively suppress T-cell proliferation

To investigate whether neutrophils acquire suppressive capacity following interaction with MSCs, we established a sequential co-culture system in which MSCs were first incubated with purified neutrophils (Supplementary Fig. 1A and 1B). MSCs were subsequently removed from the culture (Supplementary Fig. 1A and 1C and Supplementary Material Figure S1) and the MSC-conditioned ("programmed") neutrophils were then introduced into an autologous T-cell proliferation assay following polyclonal activation with anti-CD3 and anti-CD28 antibodies. Control neutrophils were used in parallel co-cultures.

MSC-programmed neutrophils induced a marked suppression of T-cell proliferation compared with control neutrophils (Fig. 1A and Supplementary Material Fig. 1). This inhibitory effect was most pronounced on day 4 post-activation and remained detectable on day 7, although the magnitude of suppression was consistently greater at the earlier time point (Fig. 1A and Supplementary Fig. 2A and Supplementary Material Fig. 1 and S2). Importantly, T-cell viability was comparable between cultures containing MSC-programmed neutrophils and those containing control neutrophils (Fig. 1B and Supplementary Fig. 2B and Supplementary Material Fig. 1 and S2), indicating that the reduced proliferative response was not attributable to increased T-cell death but rather reflected an active suppressive mechanism. Notably, neutrophils that engulfed MSCs exhibited enhanced survival compared with neutrophils cultured alone and maintained expression of key surface markers, including CD16, which was otherwise progressively lost in neutrophils cultured in isolation (Supplementary Fig. 3A and 3B and Supplementary Material Figure S3). These data indicate that interaction with MSCs not only results in MSC uptake but also confers functional benefits to neutrophils.

Importantly, the suppressive effect was heterogeneous at multiple levels. Neutrophils programmed by different MSC donors exhibited variable suppressive effects on the same T-cell donor population, indicating donor-dependent differences in the capacity of MSCs to program immunosuppressive neutrophils (Fig. 1C and supplementary Fig. 2C and Supplementary Material Fig. 1 and S2). Conversely, neutrophils from different donors conditioned by the same MSC donor displayed variable suppressive effects on autologous T cells, indicating neutrophil donor-dependent differences in responsiveness to MSC-mediated programming (Fig. 1D and Supplementary Material Fig. 1). Together, these findings highlight bidirectional inter-donor heterogeneity affecting both MSC-driven neutrophil programming and downstream T-cell responsiveness.

We next assessed whether the nature of the programming stimulus influenced the suppressive capacity of neutrophils. As mentioned above, we systematically removed MSCs from the culture (Supplementary Fig. 1A and 1C and Supplementary Material Figure S1). MSCs were first rendered necrotic by chemical treatment (acid) prior to co-culture with neutrophils (Supplementary Fig. 2D). Under these conditions, neutrophils failed to acquire the capacity to suppress T-cell proliferation, demonstrating that necrotic MSCs are unable to program neutrophils toward a suppressive phenotype (Fig. 1E and Supplementary Material Fig. 1). In contrast, neutrophils conditioned with apoptotic MSCs gained the ability to suppress T-cell proliferation, indicating that both live and apoptotic MSCs are capable of inducing a suppressive neutrophil state (Fig. 1A and 1E and Supplementary Fig. 2D and Supplementary Material Fig. 1). These

findings suggest that neutrophil programming does not strictly depend on MSC viability per se, but rather on specific interaction cues associated with MSC apoptosis or active cellular processes.

In all experiments shown in this figure, freshly isolated primary neutrophils from different donors were pre-incubated with freshly thawed MSCs for 6h or 18h. Residual MSCs were subsequently removed, and the programmed neutrophils (PR-N) or unprogrammed neutrophils (N) were co-cultured with autologous, freshly isolated T-cells (T). T-cells were labelled with CTV and then either left unstimulated (unst-T) or stimulated via CD3/CD28 stimulation (st-T). T-cell proliferation and viability was assessed by flow cytometry after 4 days of neutrophil-T-cell co-incubation.

In experiments shown in panel A, B and C of this figure, freshly isolated primary neutrophils from different donors were pre-incubated with freshly thawed MSCs for 6h. Residual MSCs were subsequently removed, and the programmed neutrophils (PR-N) or unprogrammed neutrophils (N) were co-cultured with autologous, freshly isolated T-cells (T). T-cells were labelled with CTV and then either left unstimulated (unst-T) or stimulated via CD3/CD28 stimulation (st-T). T-cell proliferation was assessed by flow cytometry after 7 days of neutrophil-T cell co-incubation.

Neutrophils engulf MSCs through an actin- and oxygen-dependent process and acquire MSC-derived material via EVs

Neutrophils are professional phagocytes, prompting us to investigate whether direct engulfment of MSCs contributes to neutrophil programming. First of all, following purified neutrophils and MSC culture over time, we quantified the recoverable MSCs and found a marked reduction in number after 6h (Fig. 2A and Supplementary Material Fig. 2).

To better reflect physiological conditions, MSCs were co-cultured with whole human blood. (Fig. 2B and Supplementary Material Fig. 2). Given that neutrophils constitute approximately 60–70% of circulating leukocytes, this experimental setting supports the likelihood of MSC-neutrophil interactions occurring in vivo following intravenous MSC administration. Leukocyte counts were adjusted to maintain a 1:1 ratio between neutrophils and MSCs. To assess whether MSC uptake occurred through classical phagocytosis, blood samples were treated with Cytochalasin D, a pharmacological inhibitor of actin polymerization. This treatment markedly reduced MSC engulfment and served as a negative control.

To directly monitor phagocytic uptake, MSCs were labeled with pHrodo, a pH-sensitive fluorophore that remains non-fluorescent at neutral pH but becomes fluorescent upon acidification within phagolysosomes (pH ~ 4). Although from the results described in Fig. 2A 2h of co-incubation was already considered too short to detect any phagocytic activity, after 6 hours of co-incubation a substantial proportion of pHrodo-labeled MSCs were detected within neutrophils, resulting in a strong fluorescent signal consistent with active phagocytosis and subsequent phagolysosomal processing of MSCs (Fig. 2B and Supplementary Material Fig. 2). In contrast, Cytochalasin D treatment greatly

diminished the pHrodo signal, confirming the dependence of MSC uptake on actin-mediated phagocytic mechanisms. In addition to neutrophils, MSCs were also internalized by monocytes, in agreement with previous reports demonstrating monocyte-mediated clearance of MSCs [18, 39]. Collectively, these findings indicate that neutrophils efficiently engulf and process MSCs under physiologically relevant conditions, supporting a potential role for neutrophil-mediated MSC clearance in vivo.

We next investigated whether environmental conditions influenced MSC uptake by neutrophils. Exposure to hypoxic conditions markedly impaired MSC phagocytosis, demonstrating a requirement for oxygen availability and suggesting that this process is metabolically regulated. Importantly, neutrophil viability remained unchanged under hypoxia, indicating that the reduced phagocytic activity was not attributable to increased cell death but rather reflected an active inhibition of MSC uptake (Fig. 2C and Supplementary Material Fig. 2).

To determine whether MSC engulfment represented a selective process, neutrophils were also co-cultured with control HEK cells. Under these conditions, neutrophils failed to phagocytose HEK cells (Supplementary Fig. 4 and Supplementary Material Figure S4), indicating that MSC uptake reflects a regulated and selective interaction rather than a nonspecific consequence of cell-cell contact.

Given the association between MSC uptake and neutrophil function, we next examined whether the extent of phagocytosis correlated with the acquisition of suppressive activity. Notably, the amount of MSCs engulfed by neutrophils showed a significant positive correlation with their capacity to suppress T-cell proliferation (Fig. 2D and Supplementary Material Fig. 2). These findings suggest that MSC phagocytosis is linked to the functional reprogramming of neutrophils and supports the notion that MSC uptake predisposes neutrophils toward an immunosuppressive phenotype.

Freshly isolated primary neutrophils from two healthy donors were co-incubated with freshly thawed HEK cells for 6 h. At the end of the co-incubation, the number of remaining HEK cells was quantified relative to the number of neutrophils by flow cytometry and normalized to Cytochalasin D-treated samples (inhib-N). Data is presented as mean ± SD from two independent healthy donors.

MSC-derived EVs are sufficient to program neutrophils to suppress T-cell activation

As described above, MSCs were labelled with pHrodo to track the transfer of MSC-derived material to neutrophils. Intriguingly, pHrodo fluorescence was still detected in neutrophils even when phagocytosis was inhibited. This observation suggested the existence of an additional mechanism of MSC-derived material transfer that operates independently of actin-driven engulfment. (Fig. 3A and 3B and Supplementary Material Fig. 3). One potential explanation for this phenomenon is the transfer of MSC-EVs. Since EV uptake can occur through mechanisms distinct from classical phagocytosis [40], we hypothesized that MSCs release EVs that are subsequently internalized by neutrophils, thereby contributing to the pHrodo signal observed under phagocytosis-inhibiting conditions.

To investigate this possibility, pHrodo-labeled MSCs were cultured in transwell systems that physically separated MSCs from isolated neutrophils while allowing the exchange of soluble factors. Despite the absence of direct cell-cell contact, neutrophils displayed a pHrodo fluorescence shift (Fig. 3C and Supplementary Material Fig. 3), indicating transfer of MSC-derived material via soluble components. To further characterize the nature of the transferred material, conditioned media were collected from MSC monocultures, neutrophil monocultures, and MSC-neutrophil co-cultures after 2 and 6 hours of incubation. Analysis of the conditioned medium revealed the presence of previously described MSC-EV markers [36], including CD44 and CD105 (Fig. 3D), whereas these markers were absent in EVs isolated from neutrophils cultured alone.

To directly assess whether MSC-derived EVs could be transferred to neutrophils, pHrodo-labeled MSCs were cultured alone for 2 or 6 hours, after which conditioned media were collected and added to freshly isolated neutrophils. Exposure to conditioned media alone was sufficient to induce a pHrodo fluorescence shift in neutrophils, demonstrating the transfer of MSC-derived material in the absence of direct cell-cell contact (Fig. 3E and Supplementary Material Fig. 3). Removal of EVs from the conditioned medium largely abolished the acquisition of the pHrodo signal by neutrophils, demonstrating that the fluorescence shift was dependent on the presence of EVs (Fig. 3E and Supplementary Fig. 5A and Supplementary Material Fig. 3 and S5). Importantly, EV uptake by neutrophils occurred rapidly, with more than 80% of EV-associated signal detected within 2 hours, substantially faster than MSC phagocytosis, which remained limited at this early time point (Fig. 3B and Supplementary Material Fig. 3).

Collectively, these data demonstrate that neutrophils interact with MSCs through two distinct but complementary mechanisms: (i) actin- and oxygen-dependent phagocytosis of whole MSCs, and (ii) rapid, actin-independent uptake of MSC-derived EVs. Both pathways likely contribute to neutrophil programming and underscore the complexity of MSC-neutrophil crosstalk.

We next investigated whether MSC-derived EVs are sufficient to program neutrophils toward a suppressive phenotype using the autologous T-cell suppression assay. Neutrophils were isolated from multiple donors (each donor was run in a separate experiment to ensure reproducibility) and incubated for 6 hours either with alive MSCs or with MSC-derived conditioned medium. Following conditioning, neutrophils were washed and introduced into autologous T-cell co-culture. Neutrophils conditioned with MSC-derived conditioned medium significantly suppressed T-cell proliferation compared with control neutrophils (Fig. 3F and Supplementary Material Fig. 3). Suppression mediated by conditioned medium-treated neutrophils was consistently observed but was less pronounced than that induced by neutrophils conditioned through direct contact with MSCs (Fig. 1A, Fig. 3F and G and Supplementary Material Fig. 3). While differences in EV concentration or exposure efficiency cannot be excluded, these findings nonetheless demonstrate that MSC-derived EVs are sufficient to confer suppressive capacity to neutrophils.

Temporal analysis revealed donor-dependent heterogeneity in the durability of the suppressive phenotype. By day 7, suppression mediated by EV-conditioned neutrophils was lost in most donors

(Supplementary Fig. 5B and Supplementary Material Figure S5). In contrast, neutrophils conditioned through direct interaction with MSCs exhibited robust and sustained suppressive activity across all donors, remaining significant at day 7 (Supplementary Fig. 2A and C and Supplementary Material Figure S2).

Collectively, these data indicate that both MSCs and MSC-derived EVs can program neutrophils to acquire immunosuppressive properties. Direct MSC-neutrophil interactions induce a more potent and durable suppressive phenotype, whereas EV-mediated programming is sufficient but appears more transient and variable across donors.

T-cell suppression by MSC-programmed neutrophils requires cell–cell contact and depends on the programming stimulus

To further define the mechanism of action of neutrophils programmed by MSCs, we investigated which stages of T-cell activation were affected by MSC-conditioned neutrophils. Since T-cell activation is characterized by the sequential expression of activation markers, with CD25 being upregulated early and HLA-DR appearing at later stages, we assessed the impact of MSC-programmed neutrophils on these activation dynamics. Co-culture with MSC-programmed neutrophils resulted in a significant reduction in surface CD25 expression on activated T-cells compared with control neutrophils, indicating T-cell activation suppression at an early stage. In contrast, later activation markers were less prominently affected, suggesting that the primary impact of MSC-programmed neutrophils occurs during the initial phases of T-cell activation (Fig. 4A and Supplementary Material Fig. 4).

We next investigated whether this effect required direct cell-cell contact. MSC-programmed neutrophils were physically separated from activated autologous T cells using a transwell system while allowing the exchange of soluble factors. Under these conditions, the suppressive effect on T-cell proliferation was completely abrogated, demonstrating that neutrophil-mediated suppression is dependent on direct cellular contact (Fig. 4B and Supplementary Material Fig. 4).

Collectively, these findings demonstrate that interaction with MSCs confers a stable, active immunosuppressive phenotype to neutrophils. This programmed state enables neutrophils to inhibit early T-cell activation and subsequent proliferation through a contact-dependent mechanism, thereby identifying neutrophils as active mediators of MSC-induced immunoregulation.

In all experiments shown in this figure, freshly isolated primary neutrophils from different donors were pre-incubated with freshly thawed MSCs for 18h. Residual MSCs were subsequently removed, and the programmed neutrophils (PR-N) or unprogrammed neutrophils (N) were co-cultured with autologous, freshly isolated T-cells (T). T-cells were labelled with CTV and then either left unstimulated (unst-T) or stimulated via CD3/CD28 stimulation (st-T). T-cell proliferation and viability was assessed by flow cytometry after 4 days of neutrophil-T-cell co-incubation.

DISCUSSION

In this study, we identify neutrophils as previously unrecognized secondary immunoregulatory effectors of MSCs and delineate the cellular mechanisms through which MSC-neutrophil interactions suppress T-cell activation. Using a robust autologous T-cell suppression platform, we demonstrate that neutrophils acquire potent, contact-dependent immunosuppressive properties towards activated T-cells following interaction with MSCs. This suppressive phenotype is predominantly immunomodulatory rather than cytotoxic and preferentially targets early T-cell activation. It is induced through complementary mechanisms involving direct MSC phagocytosis and rapid uptake of MSC-derived EVs. Together, these findings establish a mechanistic framework in which MSCs transiently reprogram innate immune cells, specifically neutrophils, to propagate adaptive immune suppression in a manner that is rapid, tunable, and context dependent.

Neutrophils have historically been viewed as short-lived effector cells with a lifespan measured in hours. However, this paradigm has been fundamentally revised by recent work demonstrating that neutrophils can survive for several days in vivo, particularly under inflammatory conditions, and can adopt phenotypically and functionally diverse states [41]. This emerging plasticity includes the concept of polarized neutrophil subsets, often referred to as N1 (pro-inflammatory) and N2 (immunoregulatory), analogous to macrophage polarization [42]. While the existence and nomenclature of such subsets remain an area of active investigation, there is increasing consensus that neutrophils can actively suppress adaptive immune responses under defined conditions. Our data place MSCs among the instructive signals capable of driving neutrophils toward a regulatory, T-cell-suppressive state.

To our knowledge, this is the first demonstration that neutrophils can directly suppress T-cell activation following interaction with MSCs. Previous studies have largely focused on MSC-T-cell or MSC-monocyte/macrophage interactions, with neutrophils often considered passive bystanders or short-lived scavengers of infused cells. In contrast, our findings position neutrophils as active intermediaries that translate MSC-derived signals into downstream adaptive immune regulation. Importantly, the suppressive effect we observe is most pronounced at early time points and wanes over time, consistent with the innate immune nature of neutrophils and their role in rapid, first-line immune modulation. This temporal profile suggests that neutrophil-mediated immunoregulation may be particularly relevant in acute inflammatory settings, such as early GvHD, cytokine storm, or acute tissue injury, where rapid dampening of T-cell activation is critical to prevent irreversible damage.

Mechanistically, we show that neutrophil-mediated suppression preferentially targets early T-cell activation, as evidenced by reduced CD25 upregulation, without affecting T-cell survival. This distinguishes neutrophil-mediated regulation from other immunoregulatory cell types that primarily interfere with late activation stages or effector differentiation, such as regulatory T cells or tolerogenic antigen-presenting cells [43, 44]. Early interception of T-cell activation may represent a particularly efficient strategy to restrain pathological immune responses while preserving immune competence, and may help explain the clinical efficacy of MSCs in acute, T-cell-driven diseases [45].

Our data further demonstrate that neutrophil-mediated T-cell suppression requires direct cell-cell contact, as separation by transwell completely abrogates the effect. This finding aligns with emerging evidence that neutrophil and T-cell interactions are highly spatially constrained and rely on synapse-like contacts, integrin-mediated adhesion, and localized delivery of inhibitory signals [26, 27, 28, 29]. Notably, soluble factors alone are insufficient to mediate suppression in this system, emphasizing that neutrophils function as contact-dependent regulators of adaptive immunity. In contrast, MSC-to-neutrophil programming can occur in the absence of direct contact, mediated in part by EVs, highlighting a division of labor in which MSCs instruct neutrophils through both contact-dependent and EV-based mechanisms, while neutrophils execute suppression via direct engagement with T-cells.

A central mechanistic insight from this work is the role of phagocytosis in neutrophil programming. We show that neutrophils actively engulf MSCs in an actin- and oxygen-dependent manner, and that neutrophils which have phagocytosed MSCs display enhanced survival and sustained expression of functional surface markers. The correlation between MSC phagocytosis and acquisition of suppressive capacity suggests that engulfment is not merely a clearance mechanism but an instructive event that reprograms neutrophil function. Interestingly, MSC phagocytosis by neutrophils occurs despite expression of CD47 [46], the canonical “don’t-eat-me” signal, indicating that MSC clearance cannot be explained by absence of inhibitory signals. In contrast to monocytes, where complement opsonization enhances MSC phagocytosis [18], neutrophil uptake of MSCs appears complement independent, pointing to distinct receptor systems that remain to be identified. Defining the receptors and signaling pathways governing MSC phagocytosis by neutrophils represents a critical next step.

In parallel to whole-cell engulfment, we identify a second, faster, actin-independent pathway by which neutrophils acquire MSC-derived material through EV uptake. EV transfer occurs within hours, precedes detectable phagocytosis, and is sufficient to confer a transient suppressive phenotype. These findings are consistent with emerging models of EV internalization that include endocytosis, membrane fusion, or passive uptake mechanisms distinct from classical phagocytosis [40]. The oxygen dependence of MSC phagocytosis, but not EV uptake, further supports the existence of mechanistically distinct entry routes. Notably, impaired phagocytosis under hypoxic conditions mirrors observations in other systems, such as reduced platelet phagocytosis by monocytes in low-oxygen environments [47, 48], underscoring the metabolic constraints governing engulfment-based immune regulation.

The differential effects of live, apoptotic, and necrotic MSCs on neutrophil programming further align this system with established paradigms in macrophage biology. Apoptotic MSCs retain the capacity to program neutrophils toward suppression, whereas necrotic or chemically inactivated MSCs do not, mirroring the well-described immunoregulatory effects of apoptotic cell clearance and reinforcing the concept that regulated cell death, rather than nonspecific debris, conveys instructive signals. This convergence suggests that MSC-mediated immune regulation may exploit conserved pathways of efferocytosis-associated tolerance across innate immune lineages.

Beyond mechanistic insight, our findings have important translational implications. The neutrophil-based T-cell suppression assay described here provides a functional, biologically relevant platform that could complement existing MSC potency assays, which are often limited to MSC-T-cell co-cultures and fail to capture indirect modes of action. Because neutrophil programming integrates both MSC properties and host immune responsiveness, this system may be particularly well suited for evaluating MSC products manufactured under GMP conditions. Moreover, by using autologous neutrophils and T-cells, this approach opens the possibility of patient-specific testing, aligning with emerging precision-medicine frameworks in which therapeutic efficacy is predicted not solely by product attributes but by the interaction between the product and the patient's immune landscape.

In this context, our work supports a broader conceptual shift in cell therapy, from a product-centric view of potency to a target-centric or patient-centric model. Neutrophils, as abundant, rapidly responsive innate cells, may serve as sensitive biosensors of both MSC quality and patient immune state. Harnessing this interaction could improve patient stratification, optimize treatment timing, and enhance therapeutic outcomes, particularly in acute inflammatory diseases where rapid immune recalibration is required.

In summary, we identify neutrophils as central, active intermediaries of MSC-mediated immunoregulation and define complementary mechanisms, phagocytosis and EV uptake, through which MSCs reprogram neutrophil function. By linking MSC clearance to innate immune reprogramming and early adaptive immune suppression, our findings provide a unifying model of MSC mode of action with direct relevance to GvHD and beyond. This work not only expands the conceptual landscape of MSC biology but also establishes a foundation for more predictive, mechanism-based evaluation of cell therapies in clinical practice.

Declarations

Ethics approval and consent to participate

All human sample collections included in this study were reviewed and approved by the Swedish Ethical Review Authority. The approvals covered the collection of an additional tube of blood from healthy blood donors during routine blood donation ("Studies of cells in human blood"; Dnr 2024-01747-01; 2024-03-11) and the collection of blood from healthy volunteers ("Mesenchymal stromal cells in preclinical research"; Dnr 2016_1582-31; 2016-10-07, amended by "Mesenchymal stromal cells in preclinical research"; Dnr 2018/1187-32; 2018-06-29). The study set up and the phagocytosis assays were conducted with MSCs isolated from 30 mL of bone marrow collected from healthy stem cell donors during bone marrow aspiration ("Study of the interaction of hematopoietic stem cells with CMV and the extracellular matrix in the bone marrow niche, and the outcome of stem cell reconstitution."; Dnr 2022-03213-02; 2022-06-08). The immunoregulation studies and the remaining experiments were performed using MSCs by Cellcolabs AB ("Basic preclinical investigation of human mesenchymal stromal cells

("MSCs") in regenerative medicine”; Dnr 2025-08474-01; 2026-05-18). All participants were fully informed about the study procedures and provided written informed consent to their participation in the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study is available as supplementary material.

Competing interests

The authors declare that they have no competing interest.

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Authors' contributions

Conceptualization, methodology and formal analysis: LT, NK, RF; Investigation: LT, KL, AG; Data curation and visualization: LT; Supervision: NK, RF, PH, KLB, JFT; Resources: NK, RF, PH, KLB, LS; Writing original draft: LT, NK; Writing, reviewing and editing: LT, KL, AG, LS, JFT, PH, KLB, RF, NK

Artificial Intelligence (AI)

The authors declare that no AI tools were used to generate, process, or analyse the experimental data.

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Figures

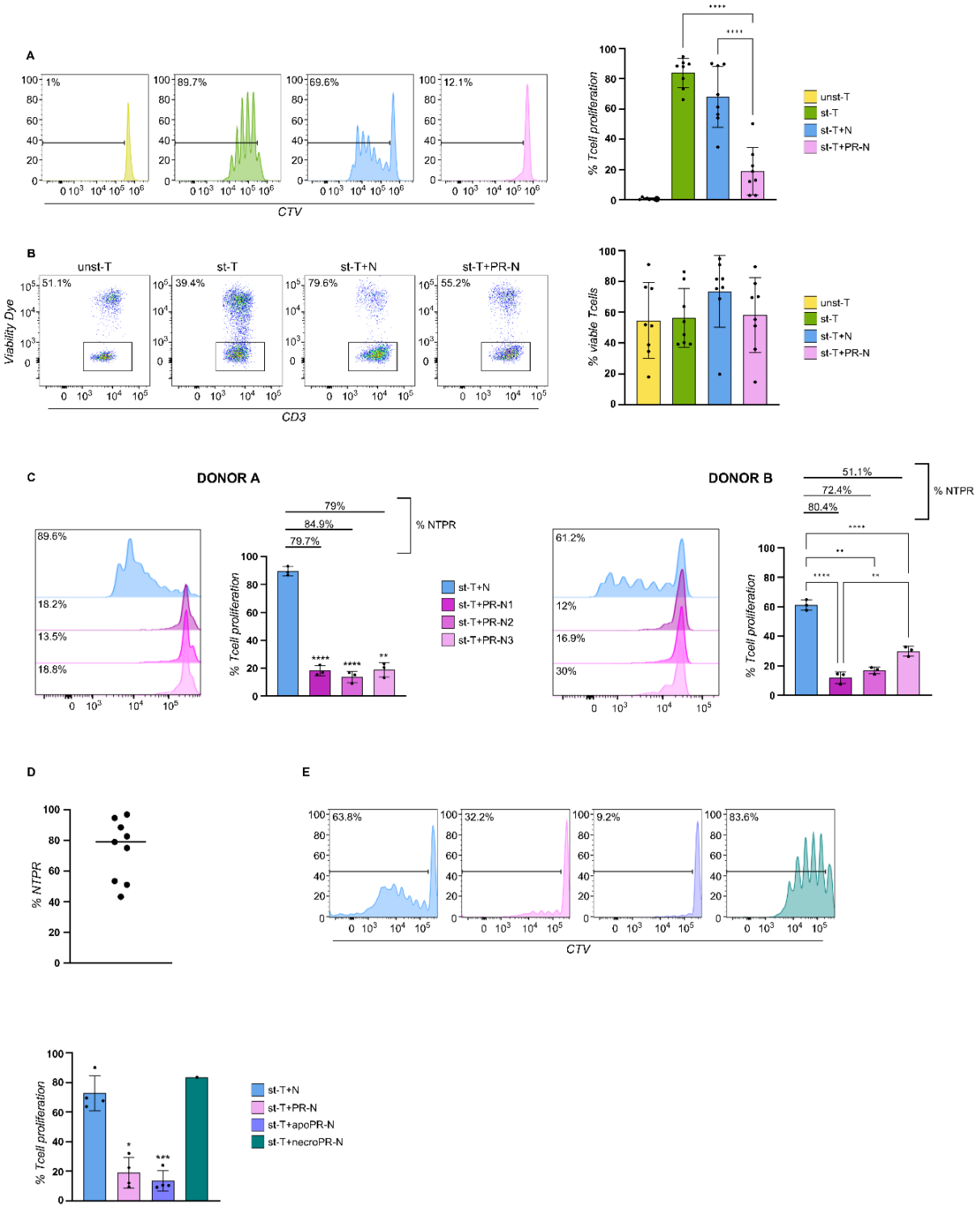


Figure 1

MSC-programmed neutrophils suppress T-cell proliferation

In all experiments shown in this figure, freshly isolated primary neutrophils from different donors were pre-incubated with freshly thawed MSCs for 6h or 18h. Residual MSCs were subsequently removed, and the programmed neutrophils (PR-N) or unprogrammed neutrophils (N) were co-cultured with autologous,

freshly isolated T-cells (T). T-cells were labelled with CTV and then either left unstimulated (unst-T) or stimulated via CD3/CD28 stimulation (st-T). T-cell proliferation and viability was assessed by flow cytometry after 4 days of neutrophil-T-cell co-incubation.

A, B) Neutrophils from multiple blood donors were pre-incubated with the same MSC source for 6h or 18h. T-cell proliferation and viability were evaluated, and the effect of MSC-mediated neutrophil reprogramming was compared to unprogrammed neutrophils, as well as to stimulated and unstimulated T-cells cultured alone. Data is presented as mean ± SD from 8 independent healthy donors. Statistical significance was assessed using one-way repeated-measures ANOVA followed by Tukey's multiple-comparisons test.

C) Neutrophils from two blood donors were each pre-incubated with three different MSC sources for 6h. The effect of MSC-mediated neutrophil reprogramming was compared to unprogrammed neutrophils. Data is representative of two independent experiments, each performed in triplicate, and are presented as mean ± SD of technical triplicates from a single blood donor. Statistical significance was assessed using one-way repeated-measures ANOVA followed by Tukey's multiple-comparisons test.

D) Median of the percentage of normalized T-cell proliferation (NTPR) in T-cells co-cultured with neutrophils from multiple donors pre-incubated with the same MSC source for 6h or 18h. Data is representative of nine blood samples from eight healthy donors used in nine independent experiments.

E) Neutrophils from multiple blood donors were pre-incubated for 18h with MSCs rendered apoptotic by heat shock (apo) or necrotic by acid treatment (necro). The effect of MSC-mediated neutrophil reprogramming was compared to unprogrammed neutrophils. Data is representative of four independent healthy donors for apoptotic MSCs and one for necrotic MSCs. Data is presented as mean ± SD. Statistical significance was assessed using one-way repeated-measures ANOVA followed by Šídák's multiple-comparisons test.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

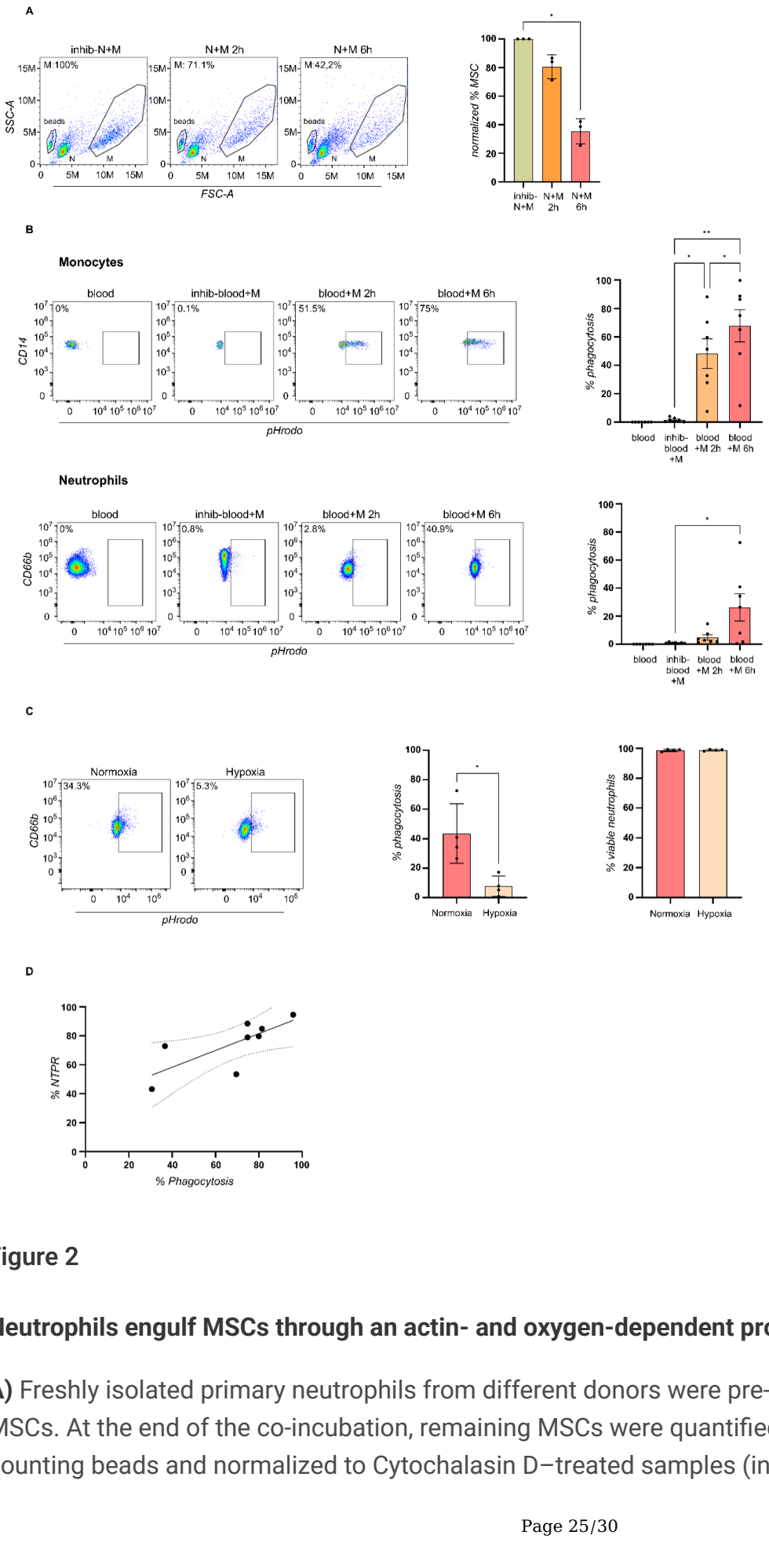


Figure 2

Neutrophils engulf MSCs through an actin- and oxygen-dependent process

A) Freshly isolated primary neutrophils from different donors were pre-incubated with freshly thawed MSCs. At the end of the co-incubation, remaining MSCs were quantified by flow cytometry by using counting beads and normalized to Cytochalasin D–treated samples (inhib-N). Data is presented as mean

± SD from 3 independent healthy donors. Statistical significance was assessed using one-way repeated-measures ANOVA followed by Tukey’s multiple-comparisons test.

B) Freshly drawn whole blood from different donors was incubated with freshly thawed MSCs labelled with pHrodo. Phagocytosis was assessed by flow cytometry based on pHrodo signal acquisition by neutrophils and monocytes following co-incubation. Gates were defined by comparing untreated blood cells (blood) to Cytochalasin D-treated samples (inhib-blood). Data is presented as mean ± SD from 7 independent healthy donors. Statistical significance was assessed using one-way repeated-measures ANOVA followed by Tukey’s multiple-comparisons test for monocytes and Šídák's multiple-comparisons test for neutrophils to focus on the comparison between inhib-blood and blood incubated with MSCs for 6h.

C) Freshly drawn whole blood from different donors was incubated with freshly thawed MSCs labelled with pHrodo. Phagocytosis was assessed by flow cytometry based on pHrodo signal acquisition by neutrophils following co-incubation. Gates were defined by comparing untreated blood cells to Cytochalasin D-treated samples (data not shown) and normoxic and hypoxic (2% O_2) conditions were compared. Neutrophil viability was additionally evaluated. Data is presented as mean ± SD from 4 independent healthy donors. Statistical significance was assessed using a two-tailed paired t-test.

D) Correlation between the percentage of phagocytosis and NTPR was determined. Phagocytosis was measured at the end of neutrophil-MSC co-incubation, and NTPR was calculated following 4 days of co-culture of neutrophils programmed by direct contact with MSCs and stimulated T-cells. Data is representative of eight independent experiments. Simple linear regression showed a significant deviation of the slope from zero ($P = 0.03$), indicating a positive linear association between the two parameters.

*$p < 0.05$, **$p < 0.01$

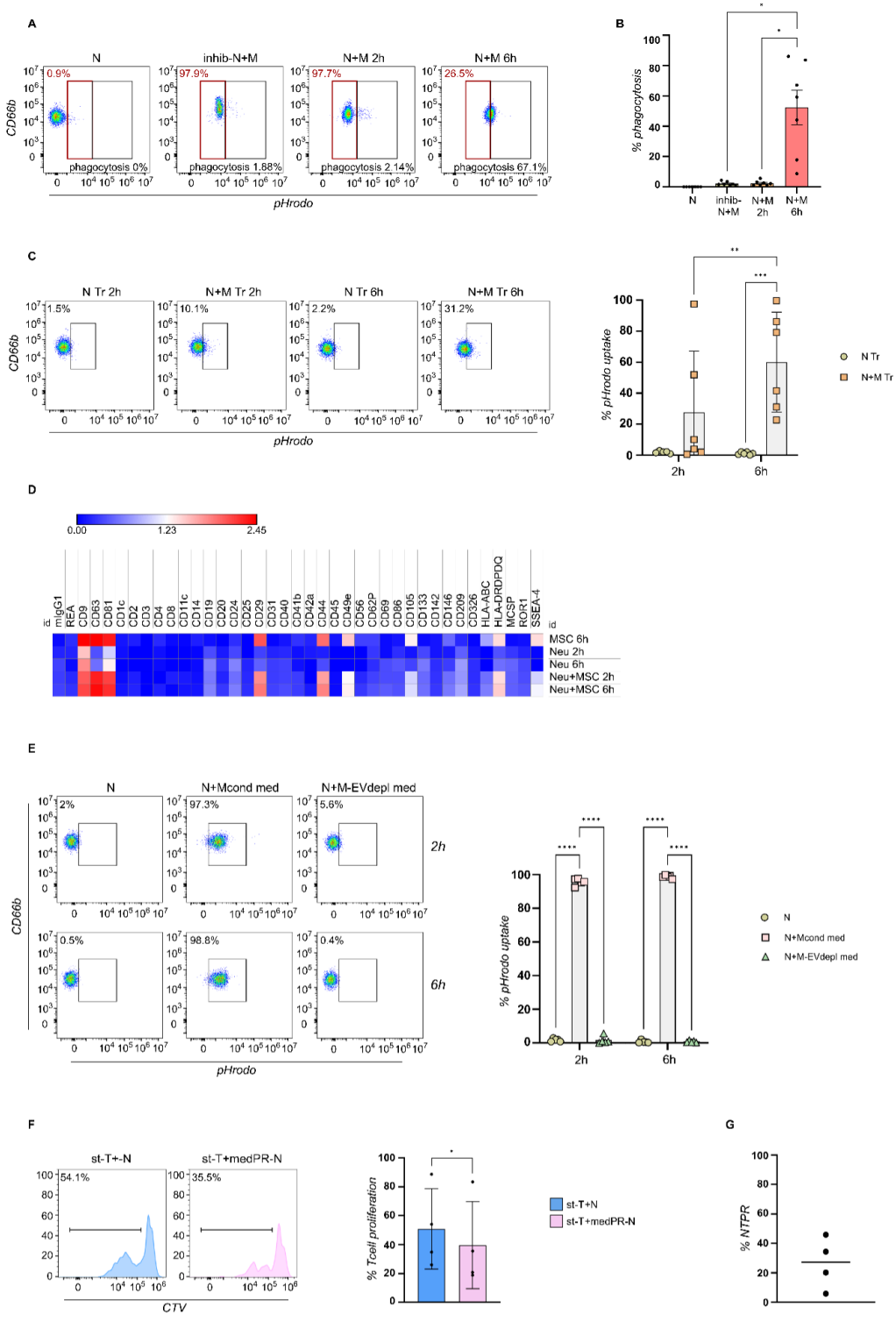


Figure 3

Neutrophils acquire MSC-derived material via extracellular vesicles and MSC-conditioned medium is sufficient to program neutrophils to suppress T-cell activation

A, B) Freshly isolated primary neutrophils from multiple donors were incubated with freshly thawed MSCs labelled with pHrodo (N+M). Based on the pHrodo signal, two gates were defined by flow

cytometry analysis: the red gate indicates phagocytosis-independent dye acquisition by neutrophils, while the black gate represents phagocytosis-dependent uptake. Phagocytosis gating was established by comparing untreated neutrophils (N) with neutrophils treated with the phagocytosis inhibitor Cytochalasin D (inhib-N). Data is presented as mean ± SD from 7 independent healthy donors. Statistical significance was assessed using one-way repeated-measures ANOVA followed by Tukey's multiple-comparisons test.

C) Freshly isolated primary neutrophils from multiple donors were incubated with freshly thawed MSCs labelled with pHrodo (N+M Tr) without direct cell-cell contact. Uptake of extracellular vesicles was assessed by flow cytometry based on pHrodo signal acquisition by neutrophils. Gates were defined using neutrophils alone (N). Data is presented as mean ± SD from 6 independent healthy donors. Statistical significance was assessed using mixed-effects model (REML).

D) Heat map illustrating extracellular vesicle signature characterized in conditioned medium obtained by neutrophils (Neu), MSCs, and neutrophil-MSC co-incubation after 2h or 6h incubations.

E) Freshly thawed MSCs were labelled with pHrodo and incubated for 2h or 6h. At the end of the incubation, conditioned medium from MSCs was collected. Half of each sample was depleted of extracellular vesicles. Freshly isolated primary neutrophils were then incubated with the conditioned (N+Mcond med) or depleted (N+M–EV_depl med) media for 2h or 6h. Uptake of pHrodo signal was measured by flow cytometry and gates were defined using neutrophils alone (N). Data is presented as mean ± SD from 3 independent experiments, where 2 healthy donors were incubated with 2 different conditioned/depleted media. Statistical significance was assessed using two-way repeated-measures ANOVA followed by Tukey's multiple-comparisons test.

F, G) Neutrophils from multiple blood donors were pre-incubated for 6h with MSC-conditioned medium. The effect of reprogrammed neutrophils (st-T + medPR-N) on T-cell proliferation was assessed by flow cytometry and compared to unprogrammed neutrophils (st-T + N) after 4 days. The percentage of NTPR was also calculated. Data is presented as mean ± SD from 4 independent healthy donors. Statistical significance was assessed using a two-tailed paired t-test.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

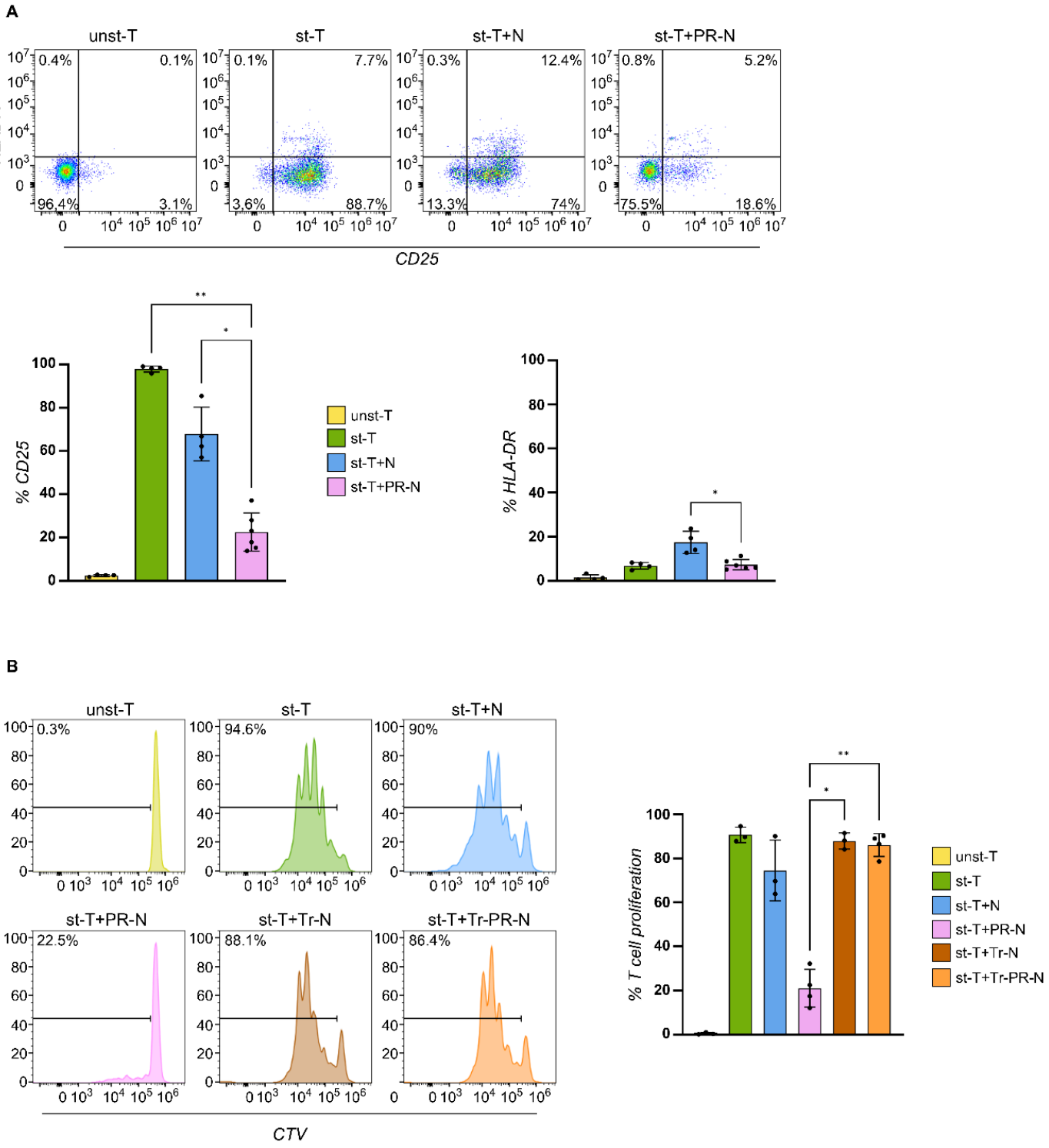


Figure 4

MSC-programmed neutrophils suppress T-cell activation at early stages and in a contact-dependent manner

In all experiments shown in this figure, freshly isolated primary neutrophils from different donors were pre-incubated with freshly thawed MSCs for 18h. Residual MSCs were subsequently removed, and the

programmed neutrophils (PR-N) or unprogrammed neutrophils (N) were co-cultured with autologous, freshly isolated T-cells (T). T-cells were labelled with CTV and then either left unstimulated (unst-T) or stimulated via CD3/CD28 stimulation (st-T). T-cell proliferation and viability was assessed by flow cytometry after 4 days of neutrophil-T-cell co-incubation.

A) Neutrophils isolated from multiple blood donors were pre-incubated with MSCs and PR-N or N were co-cultured with T-cells. Expression of CD25 and HLA-DR was assessed by flow cytometry and compared to stimulated (st-T) and unstimulated T-cells (unst-T). Data is presented as mean ± SD from 4 healthy donors and 4 independent experiments where two healthy donors were co-incubated with 2 different MSC sources. Statistical significance was assessed using mixed-effects model (REML).

B) Neutrophils from multiple blood donors were pre-incubated with MSCs. PR-N or N were co-cultured with T-cells, either in direct contact or separated by a transwell to prevent cell-cell contact (Tr-N/Tr-PR-N). T-cell proliferation was evaluated with the aim to compare the effect on T-cells by PR-N and Tr-N/Tr-PR-N by flow cytometry. Data is presented as mean ± SD from 3 independent experiments. Statistical significance was assessed using one-way repeated-measures ANOVA followed by Šídák's multiple-comparisons test.

*$p < 0.05$, **$p < 0.01$

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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- SupplementaryMaterialFigure3.xlsx
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- SupplementaryMaterialFigureS3.xlsx
- SupplementaryMateriaFigureS4.xlsx
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- SuppFigures.docx