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Tissue-Specific Stem Cells

Adipogenic Differentiation of Mesenchymal Stem Cells Alters Their Immunomodulatory Properties in a Tissue-Specific Manner

Hafsa Munir, Lewis S. C. Ward, Lozan Sheriff, Samuel Kemble, Saba Nayar, Francesca Barone, Gerard B. Nash, Helen M. McGettrick

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Abstract

Chronic inflammation is associated with formation of ectopic fat deposits that might represent damage-induced aberrant mesenchymal stem cell (MSC) differentiation. Such deposits are associated with increased levels of inflammatory infiltrate and poor progno: Here we tested the hypothesis that differentiation from MSC to adipocytes in inflamed tissue might contribute to chronicity through loss of immunomodulatory function. We assessed the effects of adipogenic differentiation of MSC isolated from bone marrow or adipose tissue on their capacity to regulate neutrophil recruitment by endothelial cells aı compared the differentiated cells to primary adipocytes from adipose tissue. Bone marr derived MSC were immunosuppressive, inhibiting neutrophil recruitment to TNFα-treate endothelial cells (EC), but MSC-derived adipocytes were no longer able to suppress neutrophil adhesion. Changes in IL-6 and TGFβ1 signalling appeared critical for the loss c

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MSC and contributes to pathogenic recruitment of leukocytes. Interestingly, stromal cells programmed in native fat tissue retain an immunoprotective phenotype. STEM CELLS *2017;35:1636–1646*

Significance Statement

Mesenchymal stem cells (MSC) can act to control inflammation, by regulating the ability of neutrophils to enter into inflamed tissues. Our data indicate that disruption of normal tissue homeostasis might drive "abnormal" MSC adipogenesis, causing the cells to lose their regulatory function. Thus, adipocytes may exist in at least two functional states: immunoprotective in healthy adipose tissue and stimulatory in sites of ectopic (e.g., chronic inflammation) fat deposition. Importantly, changes in the phenotype of MSC at sites of chronic inflammation may contribute to uncontrolled leukocyte infiltration and pathogenesis.

Introduction

Mesenchymal stem cells (MSC) are tissue-resident stromal precursors that undergo lineage-specific differentiation to repair damaged tissue and modulate a variety of immune respons 1, 2. Indeed exploiting these properties therapeutically is the principle underpinning clinical trials using MSC in chronic inflammatory diseases 1. Our recent studies revealed that MSC communicate with neighboring blood vascular endothelial cells (EC) to limit leukocyte recruitment during inflammation 3, 4. This homeostatic role is a shared characteristic of MS in a range of tissues 5. Thus, MSC act as tissue-resident regulators of leukocyte trafficking in inflamed peripheral tissue or might be delivered therapeutically to limit acute inflammatory infiltrates or to resolve chronic inflammatory disease.

Studies of the effects of lineage-specific differentiation on the immunoprotective properties MSC have been rare and have yielded conflicting results [reviewed in ref. 6]. For example, human bone marrow (BM)-derived MSC have been reported to maintain their ability to suppress the proliferation of T cells from healthy individuals or rheumatoid arthritis (RA) crosstalk with T cells 12. Comparable studies following adipogenic differentiation have not been reported. Thus it remains unclear whether differentiation of MSC adversely affects the immunoprotective functions, in particular their ability to regulate EC and leukocyte traffickir during inflammation.

Adipogenic differentiation of MSC is particularly interesting because ectopic fat deposits and/or alterations in local adipose tissue are associated with a number of inflammatory disorders including Duchenne muscular dystrophy 13, myocardial infarction 14, and type II diabetes 15. These deposits could be the result of inappropriate differentiation of tissue-resident MSC, possibly induced by inflammatory mediators in the affected tissue. Indeed platelet-derived growth factor (PDGF)receptor-alpha positive (PDGFRα$^+$) skeletal muscle MS(were identified as the source of fat deposits in a murine model of glycerol-induced muscle fibre degeneration 13. Interestingly, cardiotoxin-induced fibre degeneration did not lead to ectopic fat deposition in this muscle 13. These data suggest that aberrant adipogenic differentiation in peripheral tissues may be stimulus-specific.

Chronic inflammation has been reported to alter the phenotype of BMMSC distal to the affected site. For instance, in RA and systemic lupus erythematosus, the proliferation and senescence of BMMSC were accelerated (e.g., 7, 8, 16-18), and their osteogenic capacity was reduced 19, possibly worsening disease. What changes occur to MSC locally resident in chronically inflamed peripheral tissue is uncertain, but we have shown that tissue-resident stromal cells from chronically inflamed sites acquire a pathogenic proinflammatory phenotype, modifying EC to inappropriately recruit leukocytes 20-23. The transformed stror cells mediated their effects by altering the bioactivity of interleukin 6 (IL-6) or transforming growth factor beta 1 (TGFβ1), switching the function of these cytokines from an immunoprotective to pro-inflammatory state 20, 21, 23. Whether adipogenic differentiation MSC could drive a similar proinflammatory transformation is unknown.

The foregoing led us to test the hypothesis that aberrant differentiation of MSC to adipocyte might contribute to chronicity in inflamed tissue. We assessed the effects of adipogenic differentiation of BMMSC on their capacity to regulate neutrophil recruitment during inflammation *in vitro* and compared the differentiated cells to primary adipocytes from adipose tissue. Unlike MSC, MSC-derived adipocytes were not able to suppress neutrophil adhesion to inflamed EC. Changes in IL-6 and TGFβ1 signalling appeared critical for the loss immunosuppressive phenotype. In contrast, native stromal cells, adipocytes derived from them and mature adipocytes (mAD) from adipose tissue were immunoprotective. Thus

Isolation and Culture of Human MSC or Adipocytes

Commercially available primary human BMMSC (Lonza Ltd., Basel, Switzerland) from healthy donors were obtained at passage 2 and expanded three times in culture (i.e., to passage 5) i Mesenchymal Stem Cell Growth Medium (MSCGM) Bulletkit (Lonza Ltd., Basel, Switzerland, http://www.lonza.com/) 3-5. Based on manufacturer's information, cells had undergone 11– doublings at passage 5 and underwent approximately 2.25 population doublings per passag 5.

Adipose derived mesenchymal stromal cells (ADSC; preadipocytes) or mature adipocytes (mAD) were isolated from subcutaneous adipose tissue collected from healthy patients undergoing abdominal surgery. Tissue pieces were suspended in Dulbecco's modified Eagle medium (DMEM)/F-12 medium (Biosera, ZI du Bousquet, France, http://www.biosera.com/) supplemented with 25 mM HEPES (Sigma-Aldrich, Paisley, U.K., http://www.sigmaaldrich.cor containing 2 mg/ml collagenase type II (Sigma) at 37°C for 1 hour on a rotator. The cell suspension was filtered through a 70 μm pore filter and centrifuged at 400*g* for 5 minutes. Mature adipocytes floated and were harvested at the air–liquid interface, resuspended in DMEM/F-12 medium and cultured for 24 hours prior to use. Pelleted ADSC were resuspende in DMEM/F-12 media supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin (AD basal media; all from Sigma) and cultured as previously describ 24. ADSC were passaged once before use.

Alternatively commercially available primary human ADSC from healthy donors were obtain at passage 2 and expanded three times in culture (i.e., to passage 5) in MSC growth mediur (C-28009; all from PromoCell GmbH, Heidelberg, Germany, http://www.promocell.com/).

Differentiation of MSC into Adipocytes

BMMSC were cultured for 21 days in adipogenic induction medium (Lonza) according to the manufacturer's instructions and as previously described 5. To assess differentiation into MS derived adipocytes, samples were fixed in 10% neutral buffered formalin (Sigma) for 30 minutes, treated with 60% isopropanol (Sigma), and stained for 30 minutes with 0.3% oil rec (Sigma) dissolved in isopropanol. Samples were washed in distilled water and counterstaine with hematoxylin solution (Sigma). Cells were imaged, and digitized images were acquired using an EVOS FL Imaging System (Thermo Scientific, Loughborough, U.K., 25, converted to cDNA, and analyzed by quantitative polymerase chain reaction (qPCR) using Taqman Universal PCR Mastermix according to manufacturer's instructions (Applied Biosystems, Warrington, U.K., http://www.appliedbiosystems.com) 26. Primers for PPARγ, C/EBPα, and FABP4 were bought as Assay on Demand kits from Applied Biosystems and amplified using the 7900HT Real-Time PCR machine, analyzed using SDS 2.2 (Applied Biosystems) and expressed as $2^{-\Delta CT}$, where C_T represents the difference in the cycle number between the gene of interest and 18S (Supporting Information Fig 1B–1D).

Differentiation of Adipose Derived Preadipocytes into Adipocytes

ADSC were (5×10^5) were seeded onto inverted 0.4 μm pore Transwell filter inserts (BD Biosciences, Cowley, U.K., http://www.bdbiosciences.com/eu/home) as previously described 5 and cultured in AD basal media for 24 hours. Adipogenic differentiation was induced by culturing cells in DMEM/F-12 supplemented with 166 nM recombinant insulin, 0.2 nM triiodothyronine (T3), 33 μM Biotin, 17 μM pantothenic acid, 0.01 mg/ml transferrin, and 100 nM cortisol (all from Sigma) for 21–28 days as previously described 24. For the first 5 days, media was also supplemented with 50 μg/ml 3-isobutyl-1-methylxanthine and 2 μM Rosiglitazone (PPAR agonist) (all from Sigma).

Isolation and Culture of EC

Human umbilical vein endothelial cells (HUVEC) were isolated from umbilical cords as previously described 4, 27 and cultured in Medium 199 (Life Technologies, Paisley, U.K., http://www.lifetech.com) supplemented with 20% FCS, 35 μg/ml gentamicin, 10 ng/ml epidermal growth factor, 1 μg/ml hydrocortisone (all from Sigma), and 2.5 μg/ml Amphoteric B (Life Technologies).

Isolation of Neutrophils and Lymphocytes

Venous blood was collected from healthy donors into EDTA tubes (Sarstedt, Leicester, U.K., https://www.sarstedt.com/en/home/). Neutrophils (PMN) or peripheral blood mononuclear cells (PBMC) were isolated by centrifugation on two-step histopaque density gradients as previously described 5, 21. PBMC were panned on culture plastic for 30 minutes to remove monocytes and purify peripheral blood lymphocytes (PBL). Purified leukocytes were washed twice in PBS containing 1 mM Ca^{2+}, 0.5 mM Mg^{2+}, and 0.15% bovine serum albumin (PBSA; a from Sigma) at 250*g* for 5 minutes, counted and resuspended to 2×10^6 cells per ml in PBSA

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and cultured for 24 hours. HUVEC were then seeded on the inner surface of inserts. Cells we cocultured for 24 hours prior to treatment with or without 100 U/ml tumour necrosis factor- (TNFα; R&D Systems, Abingdon, U.K., http://www.rndsystems.com) for a further 4 hours (neutrophils) or TNFα in combination with 10 ng/ml interferon-γ (IFNγ; Peprotech Inc., Lond U.K., http://www.peprotech.com) for a further 24 hours (PBL) 3, 5, 25. Parallel endothelial monocultures were set up as controls.

In a separate series of experiments, nonadherent mAD (5×10^5 cells) were suspended in a well, on top of which a filter was placed (such that the mAD came into contact with the basa surface of the filter). Cells were cultured 24 hours prior to seeding EC into the filter as above Cocultures were established for 24 hours and cytokine treated as above.

In some experiments, a neutralizing antibody against IL-6 (5 µg/ml; clone 6708; R&D System was added when cocultures were established and was present throughout the coculture an cytokine stimulation.

To investigate the bioactivity of conditioned media, supernatants were obtained from MSC a MSC-derived adipocyte monoculture and cocultures at 24 hours. Fresh EC monocultures we treated with conditioned media for 24 hours prior to stimulation with TNFα in the same conditioned media for a further 4 hours 5.

Cocultures were also formed with collagen gels containing MSC, and EC cultured on top. Rat tail collagen type 1 (2.15 mg/ml; First Link Ltd., West Midlands, U.K., http://www.firstlinkuk.co.uk/) was mixed with 10× M199 and then neutralized by addition of N NaOH on ice, as described 21, 28, 29. MSC (2.5×10^4cells per well in a 12-well plate) were added to 500 µl, and the gel was allowed to set for 15 minutes at 37°C, and then equilibrate for 24 hours. HUVEC were seeded on the surface of the gel and cocultured with MSC for 24 hours, prior to cytokine treatment as above.

Flow-Based Adhesion Assay

Flow-based adhesion assays were performed for filters incorporated into a custom-made parallel-plate flow chamber, using phase-contrast digital microscopy as previously describe 4. Purified leukocytes were perfused over EC for 4 minutes followed by washout with cell-fre PBSA, all at a wall shear stress of 0.1 Pa. Digitized recordings of 5–10 random fields were ma 2 and 9 minutes after the end of the leukocyte bolus to assess leukocyte adhesion and proportion of the bound cells that had transmigrated was evaluated.

Collagen Gel-Based Static Adhesion Assay

Neutrophils (5×10^5 cells per ml) were added to the EC for 20 minutes, and nonadherent cel were removed by washing three times in PBSA. Neutrophil adhesion and migration was assessed using phase contrast digital microscopy by acquiring digitised z-stack images from five random fields on the surface of the endothelium throughout the depth of the gel at 2 hours, as described 29. Images were analyzed offline using Image-Pro Plus software. The number of neutrophils, adherent on the surface of the endothelium and throughout the gel was averaged per field. From the known areas of the field and well, these counts were converted to totals per gel and expressed as a percentage of the number of cells added. The proportion of the adherent neutrophils migrated into the gel was also calculated.

Gene Expression Analysis

Gene expression was analyzed using mRNA isolated from EC, MSC, and MSC-derived adipoc monocultures, or cocultures as described previously 5. Data were calculated as $2^{-\Delta CT}$ relative expression of 18S, as described above.

Quantification of Soluble Mediators

Culture supernatants were obtained from unstimulated EC and MSC cultured alone or in coculture for 24 hours. IL-6 and soluble IL-6 receptor (sIL-6R) were quantified using IL-6 Duo ELISA and sIL-6R Quantikine ELISA Kit, respectively (R&D Systems) according to manufacture instructions.

Ethics

The study was conducted in compliance with the Declaration of Helsinki. All human samples were obtained with written, informed consent and approval from the Human Biomaterial Resource Centre (Birmingham, U.K.), West Midlands and Black Country Research Ethics Committee, North East—Tyne and West South Research Ethics Committee, or University of Birmingham Local Ethical Review Committee.

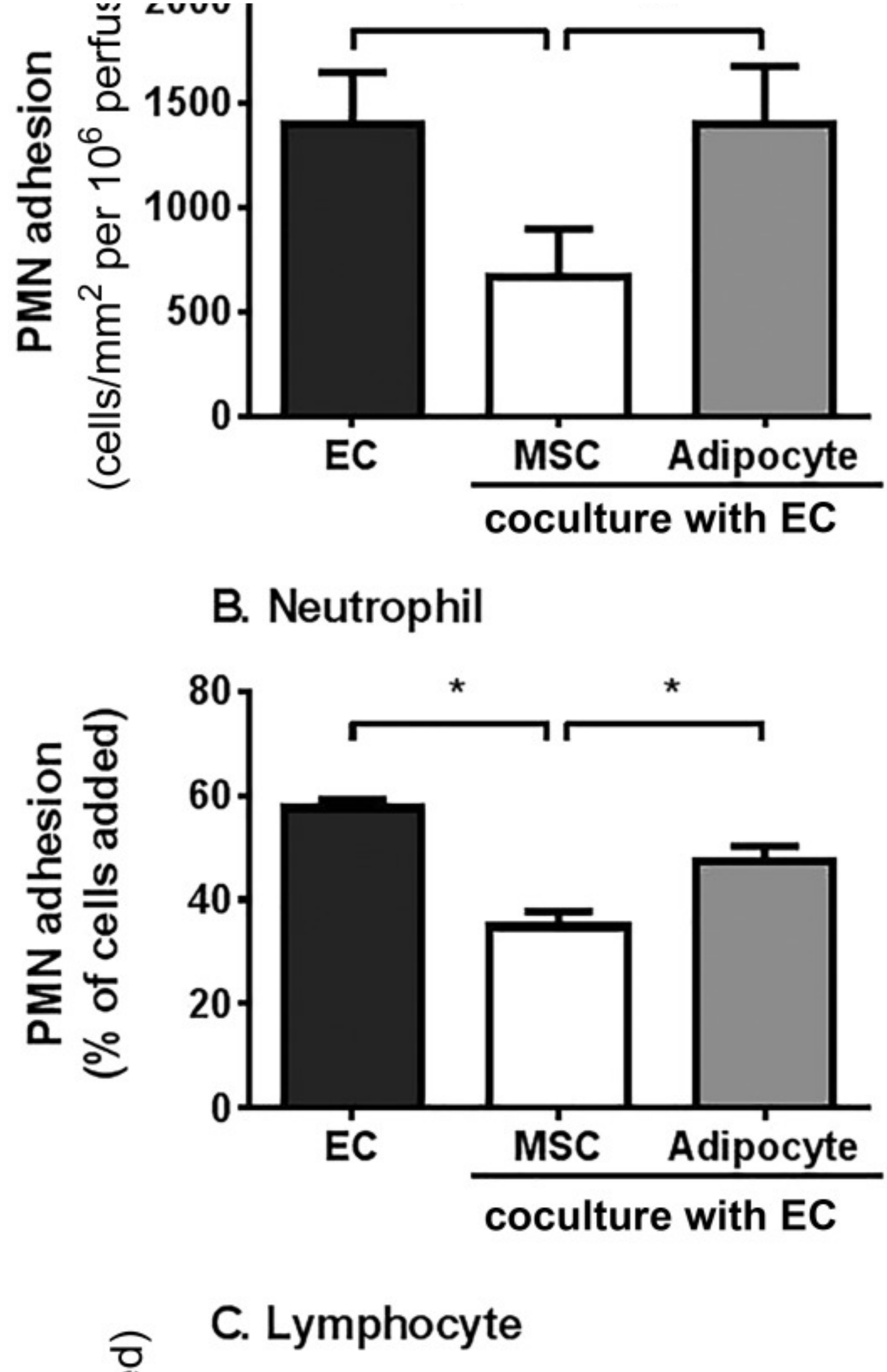
Statistical Analysis

comparisons between treatments or to controls respectively. Univariate data were analyzed using paired *t* test. $p \leq 0.05$ was considered statistically significant.

Results

Effect of Adipogenic Differentiation on the Ability of MSC to Suppre Leukocyte Recruitment by EC

MSC differentiation into adipocytes was confirmed by the presence of prominent lipid dropl and the significant upregulation of the adipocyte-related genes (PPARγ, C/EBPα, and FABP4) compared to undifferentiated MSC (Supporting Information Fig. 1). In coculture with EC, BMMSC suppressed neutrophil adhesion to TNFα-stimulated EC under flow conditions (Fig. and in a static 3D tissue model (Fig. 1B). This effect was lost when BMMSC-derived adipocyte were incorporated into coculture, such that they no longer inhibited neutrophil recruitment from flow (Fig. 1A) or adhesion in the 3D model (Fig. 1B). Similar effects were observed wher trabecular bone or umbilical cord MSC, or MSC-derived adipocytes were cocultured with EC (Supporting Information Fig. 2; also see Supporting information for derivation of MSC). Lymphocyte recruitment to TNFα and IFNγ-stimulated EC was suppressed by BMMSC, but ir this case, MSC-derived adipocytes continued to suppress recruitment when incorporated in the cocultures (Fig. 1C). Adipogenic differentiation of MSC had no significant effect on the proportion of adherent leukocytes migrating through the endothelium when compared to tl undifferentiated MSC cocultures or the EC cultured alone (data not shown). Thus, MSC lost their immunosuppressive effects on neutrophil, but not lymphocyte, recruitment upon differentiation into adipocytes. These responses were shared by MSC from several tissues. A subsequent experiments were performed using MSC isolated from BM.


PMN adhesion
(cells/mm2 per 10^6 perfus
1500
1000
500
0
EC
MSC
Adipocyte
coculture with EC
B. Neutrophil
80
60
40
20
0
*
*
PMN adhesion
(% of cells added)
EC
MSC
Adipocyte
coculture with EC
C. Lymphocyte

released in different cocultures, hypothesizing that they might account for the differences ir effects of MSC and MSC-derived adipocytes on neutrophil adhesion. MSC-derived adipocyte monocultures released significantly more IL-6 compared to undifferentiated MSC monocultures (Fig. 2A). MSC cocultures released a higher concentration of IL-6 than MSC monocultures, but production of IL-6 did not increase when MSC-derived adipocytes were cocultured with EC (Fig. 2A). As a result, the two types of coculture generated similar levels c IL-6. sIL-6R levels were also similar between all culture conditions tested (Fig. 2B). Thus, loss MSC-mediated suppression upon adipogenic differentiation was not due to a decrease in th release of IL-6 or its receptor in MSC-derived adipocyte cocultures. This raised the question whether IL-6 present in the coculture secretome remained bioactive.

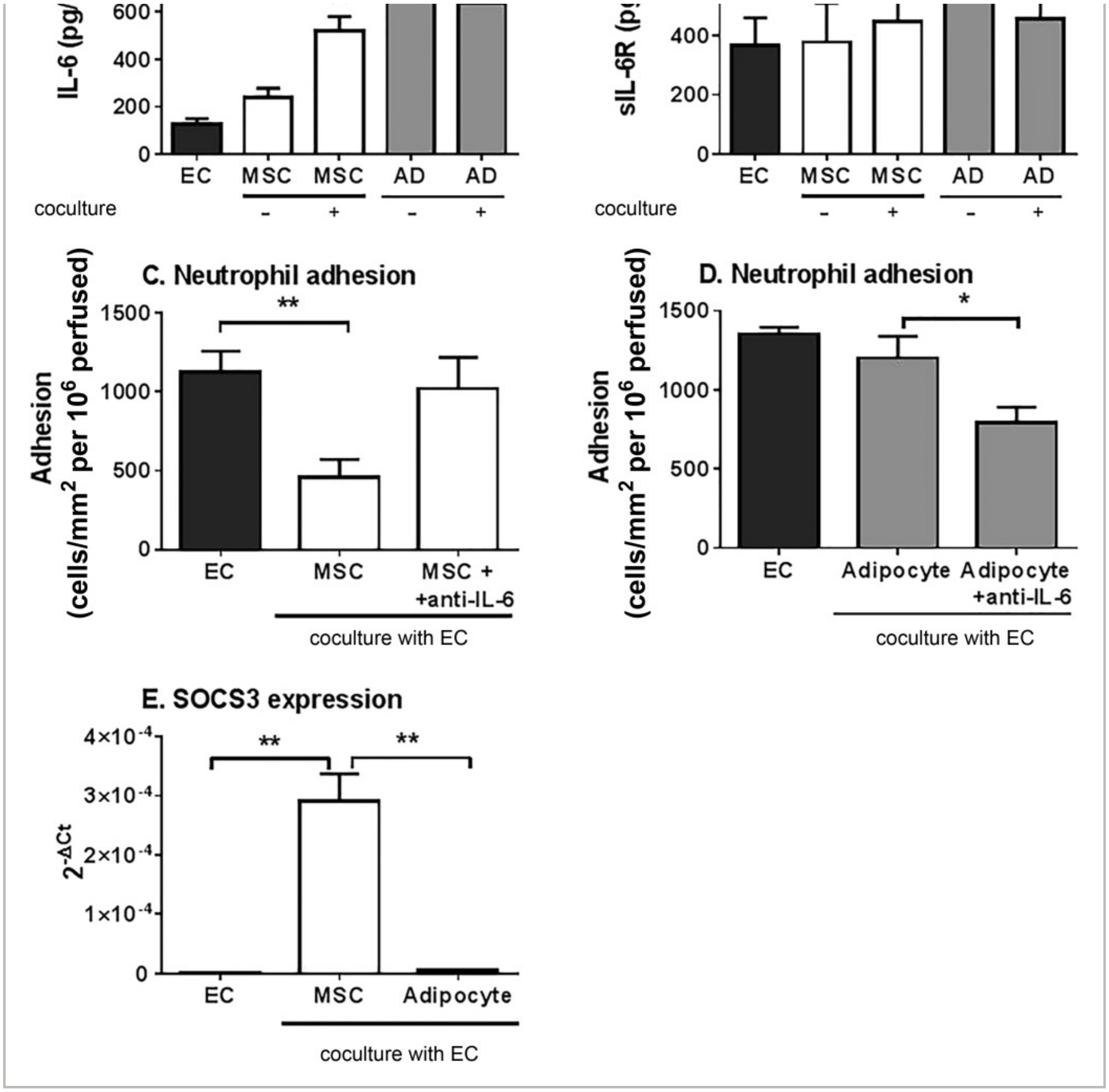


Figure 2

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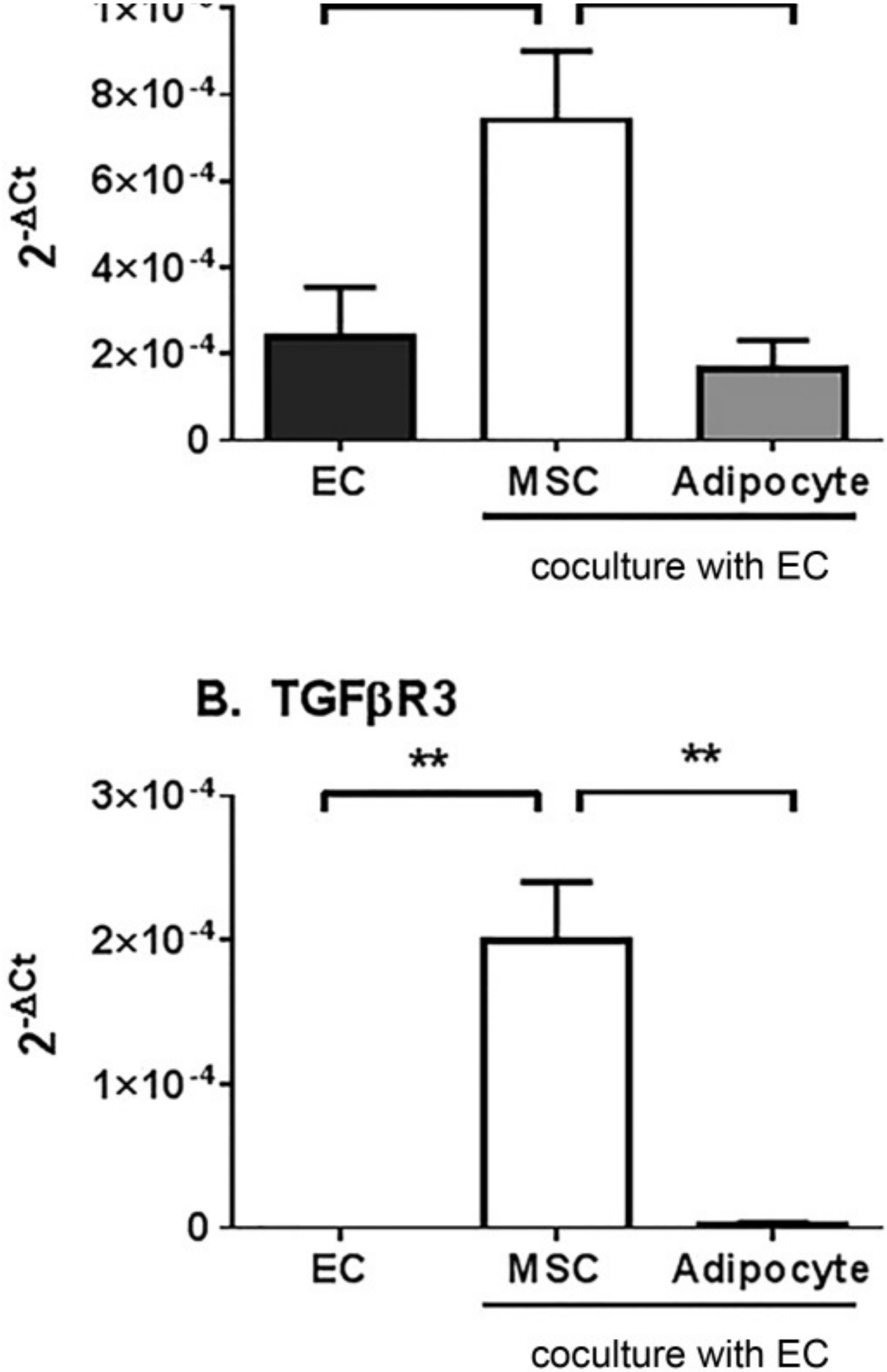
Secretion and role in immunosuppression of IL-6 in cocultures of MSC or MSC-derived adipocytes. **(A)** IL-6 and **(B)** sIL-6R release into supernatants from EC, bone marrow MSC (BMMSC), and BMMSC-derived adipocyte monoculture and cocultures was assessed after 24 hours. **(C)** BMMSC or **(D)** BMMSC-derived adipocyte cocultures were treated with neutralizing antibodies against IL-6 for the duration of the coculture and cytokine treatment. Neutrophil adhesion was assessed and expressed as the number of cells adherent/mm^2 per 10^6

coculture (Fig. 2C). In contrast, neutralization of IL-6 in MSC-derived adipocyte cocultures significantly reduced neutrophil adhesion (Fig. 2D), restoring immunoprotective function. Th IL-6 had essentially opposite effects in cocultures with either MSC or MSC-derived adipocyte supporting the concept that conversion of MSC to adipocytes resembled a pathological process.

Suppressor of cytokine signaling 3 (SOCS3) is an IL-6 inducible gene known to regulate TNFα responses [reviewed in ref. 31]. Here, expression of SOCS3 in endothelial cells was upregulated by coculture with MSC, but not MSC-derived adipocytes, when compared to the EC monoculture controls (Fig. 2E). Thus, downstream signaling from IL-6/sIL-6R differed between the two forms of coculture.

Role of TGFβ1 Signalling in Variations in Immunosuppression

We have previously reported a role for $TGFβ_1$ in mediating the immunosuppressive actions MSC in coculture 3. We wondered whether MSC differentiation might alter $TGFβ_1$ responses our cocultures. Following coculture with MSC, EC expressed significantly higher levels of TGF R_1 and TGFβ-R_3 at the transcript level compared to the EC monoculture controls (Fig. 3). In contrast, MSC-derived adipocytes had no effect on the expression of these TGFβ receptors b EC (Fig. 3). Thus loss of $TGFβ_1$ downstream signaling may contribute to the changes in IL-6 signaling and loss of immunosuppression in MSC-derived adipocyte cocultures.


8×10-4
6×10-4
4×10-4
2×10-4
0
2-ΔCt
EC
MSC
Adipocyte
coculture with EC
B. TGFβR3
**
**
3×10-4
2×10-4
1×10-4
0
2-ΔCt
EC
MSC
Adipocyte
coculture with EC

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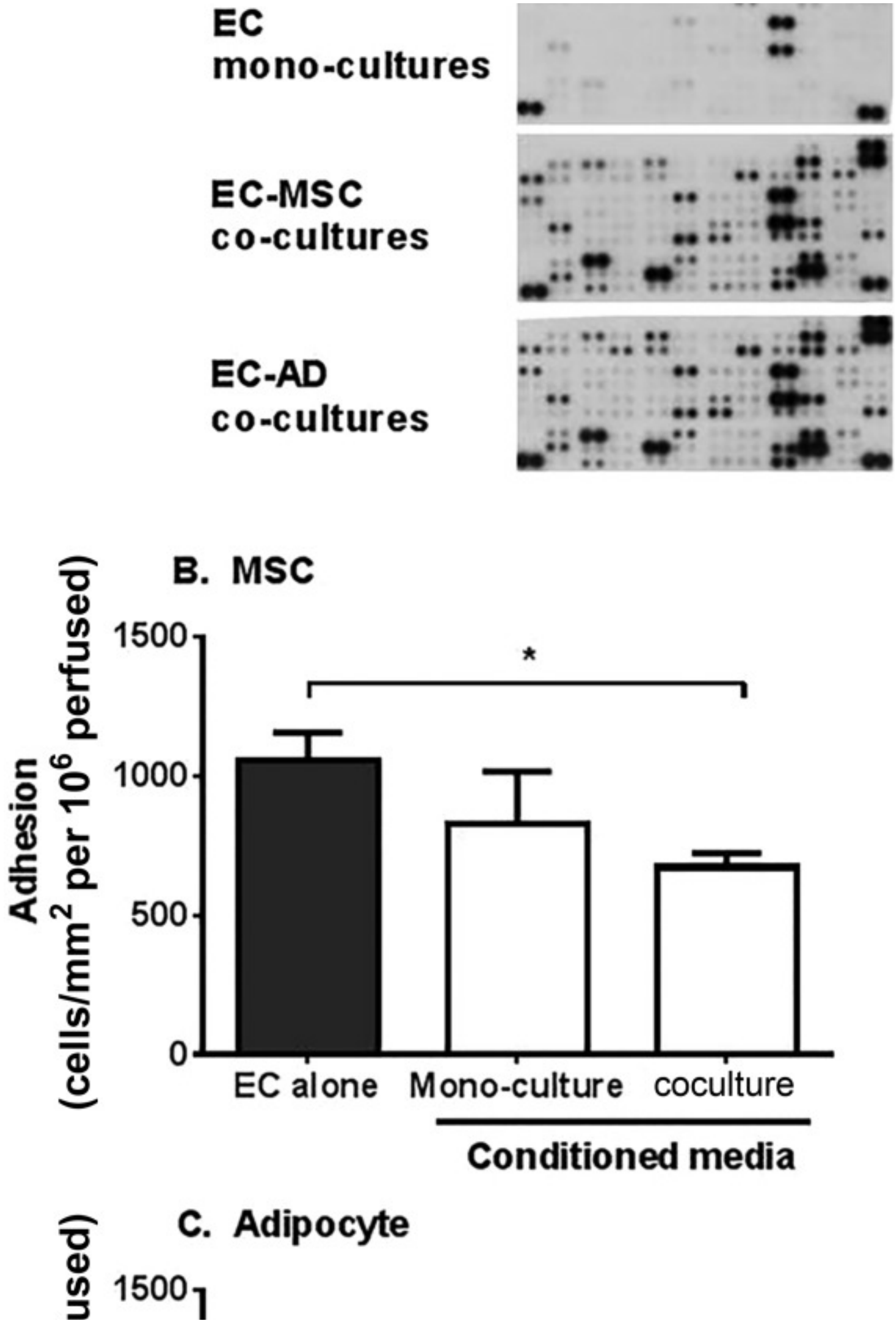
a cytokine expression array (see Supporting Information for methods), we detected higher levels of some analytes (e.g., angiogenin, CD105, and platelet derived growth factor (PDGF)-/ and lower levels of others [e.g., Dickkopf-related protein 1, CXCL12, vascular endothelial growth factor (VEGF)] in the MSC-derived adipocyte coculture supernatants when compared the MSC cocultures (Fig. 4A; Supporting Information Fig 3). In some cases, the expression of analytes, such as adiponectin, resistin, or IL-6, was comparable between both coculture conditions (Supporting Information Fig. 3). We thus tested whether the MSC-derived adipoc coculture secretome had different bioactivity compared to MSC in our recruitment assays. MSC coculture conditioned media mimicked the effect of MSC-EC coculture, inhibiting neutrophil adhesion to TNFα-treated EC monocultures *in vitro* (Fig. 4B), as previously describ 5. In contrast, conditioned media from MSC-derived adipocyte monoculture or coculture ha no effect on neutrophil adhesion (Fig. 4C). This indicates that differentiation of MSC into adipocytes alters both the composition and bioactivity of the secretome generated during coculture. Thus raises the possibility that other bioactive soluble agents generated by MSC-derived adipocyte cocultures are capable of modulating IL-6 and $TGF\beta_1$ responses, such tha they are no longer immunoprotective.


EC
mono-cultures
EC-MSC
co-cultures
EC-AD
co-cultures
B. MSC
Adhesion
(cells/mm² per 10⁶ perfused)
1500
1000
500
0
*
EC alone
Mono-culture
coculture
Conditioned media
C. Adipocyte
used)
1500

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from the tissue exerted the same effects as BMMSC-derived adipocytes on leukocyte recruitment to EC. Coculture with ADSC significantly reduced neutrophil recruitment to inflamed EC (Fig. 5A), sharing the immunosuppressive capabilities seen in MSC from other tissue sources 3, 5. Unlike BMMSC-derived adipocytes, both ADSC-derived adipocytes and mAD were immunosuppressive in coculture, significantly inhibiting neutrophil adhesion to TNFα-stimulated EC (Fig. 5B, 5C). Suppression of adhesion was also evident when lymphocyt were perfused over these cocultures (Fig. 6). We also detected high levels of IL-6 in ADSC an mAD monocultures, which did not increase when these cells were cocultured with EC (Supporting Information Fig. 4). However, in contrast to BMMSC-derived adipocytes, neutralization of IL-6 significantly inhibited the immunosuppressive effects of ADSC in coculture (Fig. 5D). Thus adipocytes derived from adipose tissue stromal cells and “native” adipocytes maintained immunoprotective effects, while adipose cells derived from BMMSC lost this capability. It appears, therefore, that ectopic MSC-derived adipose cells would lack t ability of 'true' adipose tissue derived stromal cells to regulate leukocyte recruitment.

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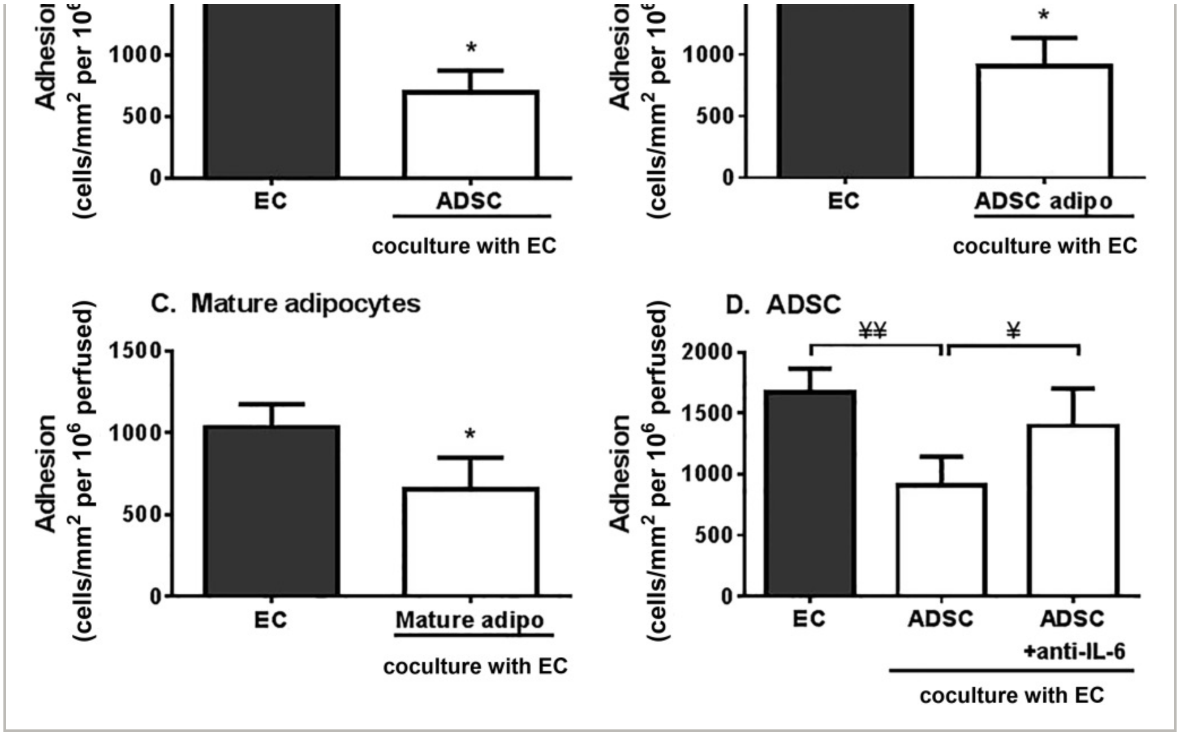

Figure 5

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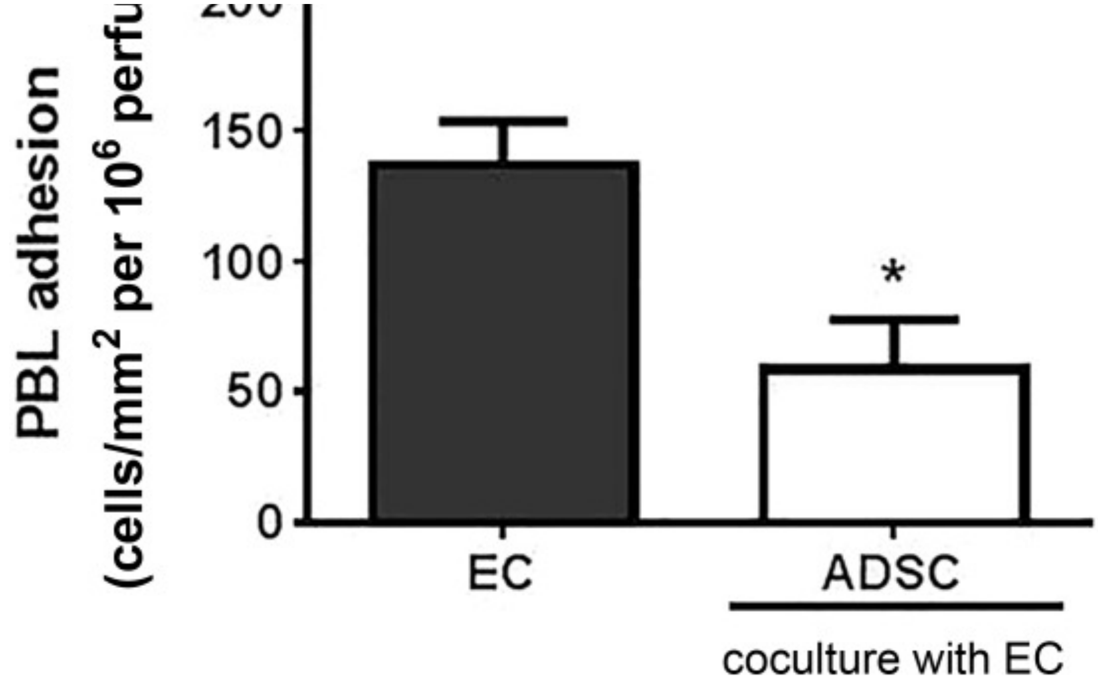
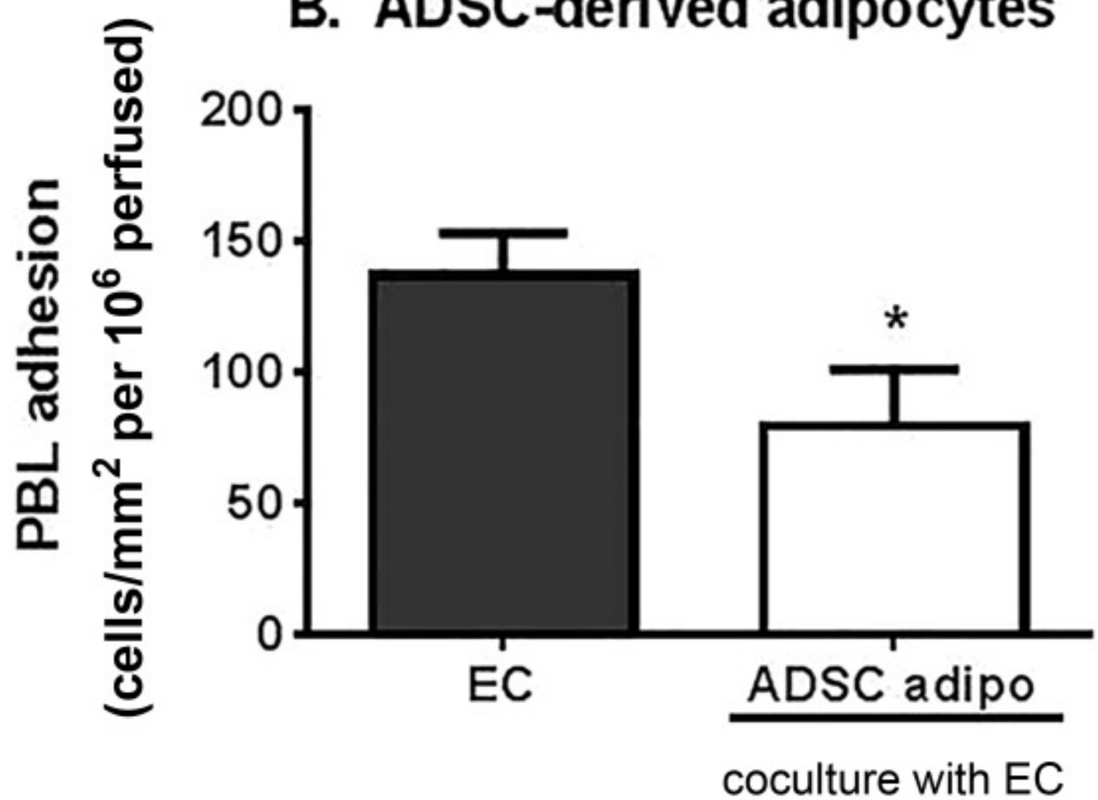
Immunosuppressive effects by ADSC and adipocytes on neutrophil recruitment. EC were cocultured with **(A)** ADSC, **(B)** ADSC-derived adipocytes (ADSC adipo) or **(C)** mature adipocytes (Mature adipo) for 24 hours prior to stimulation with tumor necrosis factor α (TNFα) for 4 hours. **(D)** ADSC cocultures were treated with neutralizing antibodies against IL-6 for the duration of the coculture and cytokine treatment. Neutrophil adhesion was assessed at 2 minutes postperfusion and expressed as the number of cells adherent/mm^2 per 10^6 cells perfused. In (D), ANOVA showed a significant effect of treatment on neutrophil adhesion, $p < 0.05$. Data are mean ± SEM, (A) $n = 6$, (B) $n = 3$, and (C, D) $n = 4$ independent experiments using a different EC and neutrophil donor in each experiment. Three different stromal cell donors were used in all experiments, except (D) where one donor was used. *, $p < 0.05$ compared to EC monoculture by paired *t* test. ¥, $p < 0.05$ and ¥¥, $p < 0.01$ by Tukey post-test. Abbreviations: ADSC, adipose-derived stromal cells; EC, endothelial cells; IL, interleukin.

Caption



B. ADSC-derived adipocytes



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adipogenesis on MSC crosstalk with EC and regulation of the inflammatory infiltrate. We als compared this form of immunosuppression between adipocytes generated from adipose tissue, and adipocytes derived from MSC from other tissues. Interestingly, they were not the same. Upon coculture with EC, BMMSC-derived adipocytes had lost the ability of BMMSC to suppress neutrophil adhesion. The ability to suppress lymphocyte adhesion was retained. T phenotype was shared with adipocytes differentiated from MSC derived from other non-adipose tissue. In contrast, adipocytes differentiated from adipose-derived stromal cells anc mature adipocytes retained immunoprotective capacity, suppressing both neutrophil and lymphocyte recruitment. Thus the effect of adipogenesis on immunoregulation appears to b tissue specific, with stromal cells from adipose and nonadipose tissues generating cells with different capabilities.

A key attribute of MSC is their capacity to differentiate into various cell lineages to aid the tissue repair processes. However, we understand little about how this process affects MSC behavior, or if the context in which differentiation is triggered can alter the outcome. Here, MSC isolated from non-adipose tissue lost some of their immunoprotective effects following adipogenesis, such that they were no longer able to inhibit neutrophil adhesion. This characteristic was not shared by ADSC, which retained their immunomodulatory capacity ev after differentiation. Of note, the latter observation argues against the loss of suppression upon adipogenesis by non-adipose-derived MSC being an artefact of in vitro differentiation, in vitro differentiation of ADSC had no effect on their suppressive potential.

IL-6 is known to have different functions, eliciting immunoprotective responses or proinflammatory effects, depending on the inflammatory and stromal context [reviewed in 32]. Indeed here and in our previous studies 3, 5, IL-6 was identified as a bioactive agent required for the inhibitory effects of MSC in coculture. However, neutralization of IL-6 revers the effects of MSC-derived adipocytes in coculture to be inhibitory, suggesting that under these conditions, IL-6 had not just lost efficacy but triggered stimulatory rather than inhibitc signals. These findings are akin to those reported for synovial fibroblasts from patients with rheumatoid arthritis in coculture with EC, where IL-6 signaling stimulated recruitment of leukocytes 20, 21. Collectively these data support the concept that conversion of MSC to adipocytes resembles a pathological process. Here, we found that in MSC cocultures, IL-6 induced the expression of SOCS3 in EC, a typically IL-6-regulated gene known to downregulɛ cellular responses to cytokines 31, and which we previously found to play a role in

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manner and elicit different functional consequences in EC.

To the best of our knowledge only two studies have assessed MSC following adipogenesis, a neither observed any alterations in their ability to inhibit T-cell proliferation 9 or dendritic ce maturation 12. Similarly, suppression of T-cell proliferation remained unaffected by osteoge differentiation of human BMMSC 7-9 or ADSC 8. On the other hand, MSC-derived chrondocytes have been reported to inhibit 9, 10 or have no effect on 11, 12 on T-cell responses. In the latter studies, reduction in bioactive soluble mediators, such as nitric oxid or prostaglandin E2, were linked to the defect in suppressive activity of MSC after differentiation 11. Here we also observed a different profile of soluble mediators released b MSC and differentiated MSC in coculture. Moreover, conditioned medium from MSC-derivec adipocyte cocultures was unable to suppress neutrophil recruitment in the same manner as medium generated from MSC cocultures. This clearly shows that the secretomes generated crosstalk between EC and MSC or MSC-derived adipocytes have different bioactivity. Thus w propose that the changes in the bioactivity of IL-6 in MSC-derived adipocyte cocultures occu due to the presence of additional soluble mediators (exclusive to these cocultures) that caus a reduction in TGFβ receptors and thus response to $TGF\beta_1$, along with a failure to induce the suppressor genes SOCS3, usually associated with IL-6 stimulation. Further work is required t identify the exact soluble mediator(s) responsible for these changes, which have the potenti to be novel therapeutic targets for diseases involving ectopic fat deposition.

Healthy adipose tissue is considered to be intrinsically immunoprotective, largely through th abundant production of the immunosuppressive adipokine, adiponectin. Indeed our data would agree with this, with stromal cells at all stages of lineage commitment (preadipocytes differentiated adipocytes, mAD) exhibiting immunosuppressive effects in our assays. Moreover, adipose-derived MSC are known to inhibit T-cell proliferation [reviewed in Ref. 34 and appear to retain this capacity after differentiation into osteoblasts 7, 8 or adipocytes 35 Thus adipocytes, much like stromal cells from other healthy tissues 5, 21, 36, have a homeostatic role in limiting inflammatory leukocyte infiltration. Indeed, IL-6 is the shared common bioactive immunoprotective mediator of healthy stromal cells 5, 21, 36 and was responsible for the immunoprotective capacity of adipose-derived stromal cells in coculture Here, MSC-derived adipocytes, ADSC, and mAD all secreted high levels of IL-6 in culture, whi were not altered by coculture with EC. Given the different effects of these cocultures, clearly is not simply the concentration of IL-6 but also the downstream signalling pathway(s) it elicit which are critical for its anti-inflammatory effect. Further work is required to determine the

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with chronic inflammatory diseases. Therefore, it may not be surprising that the endogenou immunoprotective effect of the tissue stroma may become distorted. Supporting this conce neutrophil infiltration into intra-abdominal adipose tissue was increased in mice on a high-f diet compared to those on a normal chow diet 37. Thus adipocytes may have the potential t exist in at least two functional states: (a) immunosuppressive as found in healthy adipose tissue and (b) stimulatory in sites of abnormal (obesity) or ectopic (chronic inflammation) fat deposition. Whether the adipocytes from the latter two sites are phenotypically and functionally the same remains to be determined. Our work also suggests an unexplored scenario; that changes in the phenotype of MSC at sites of chronic inflammation may contribute to uncontrolled leukocyte infiltration and pathogenesis.

Conclusion

In their natural environment MSC act to endogenously dampen EC responses to cytokines, thereby limiting leukocyte recruitment in an IL-6 dependent manner. However, aberrant differentiation of non-adipose-derived MSC causes the cells to lose this intrinsic immunoprotective capacity. Alterations in the phenotype and function of MSC may take the brake off and contribute to pathogenic inflammatory responses by altering the way in which IL-6 and TGFβ_1 exerts their effects, switching from anti-inflammatory to pro-inflammatory. T does not appear to be a characteristic of adipose tissue per se but may occur when MSC differentiate in an inappropriate location or circumstance.

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Disclosure of Potential Conflicts of Interest

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Supporting Information

References

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