

Supplementary Materials for
Human gingival mesenchymal stem cells retain their growth and immunomodulatory characteristics independent of donor age

Jay R. Dave *et al.*

Corresponding author: Geetanjali B. Tomar, geetanjalitomar13@gmail.com

Sci. Adv. **8**, eabm6504 (2022)
DOI: 10.1126/sciadv.abm6504

The PDF file includes:

Figs. S1 and S2
Tables S1 to S3
Legend for movie S1

Other Supplementary Material for this manuscript includes the following:

Movie S1

SUPPLEMENTARY MATERIALS

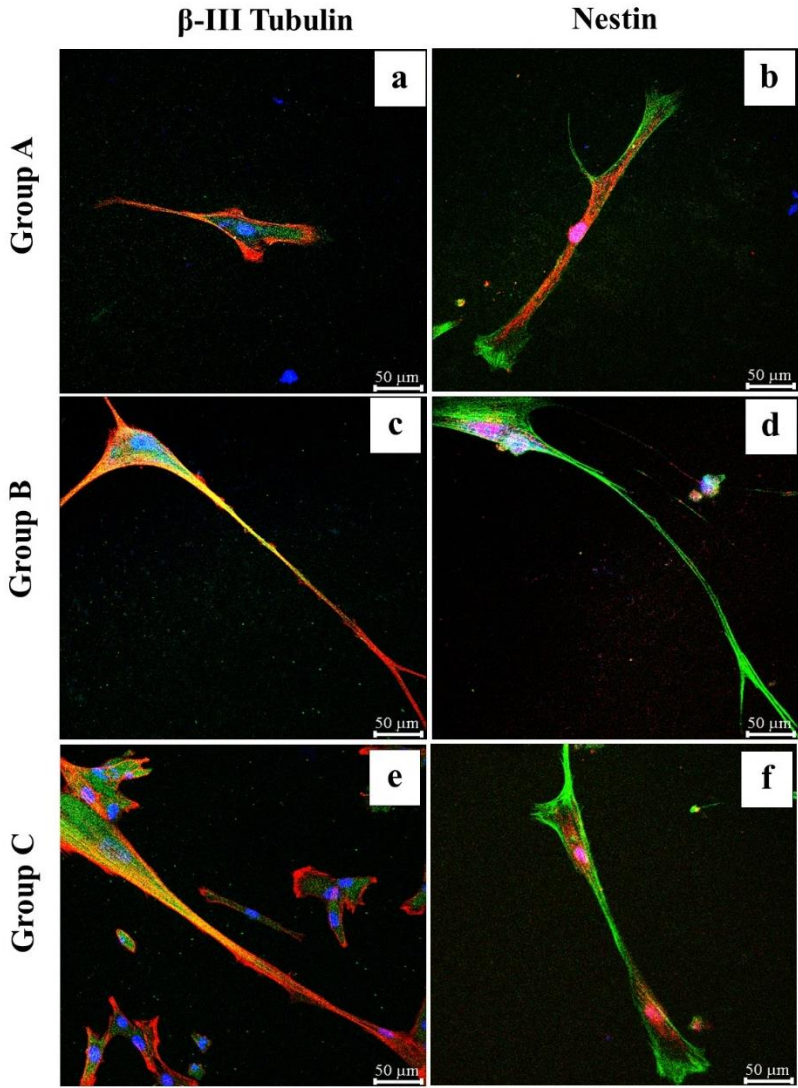


Fig. S1. Neuronal differentiation of GMSCs: Upon neurogenic induction GMSCs from all the groups displayed similar expression of β -III tubulin (a, c and e) (green; phalloidin – red; DAPI

– blue nuclei) and nestin (b, d and f) (red; phalloidin – green; DAPI – blue nuclei). (Magnification 40x oil; scale 50 μ m). The cell size in Group B GMSCs was larger as compared to Group A and Group C after neuronal differentiation.

Method: In order to study an age-appropriate effect of GMSCs in animal model of ALI, we also induced ALI in young (6-8 week), adult (15-18 week) and elderly (27-30 week) Swiss albino male mice by intra-nasal administration of LPS at a concentration of 5 μ g/gm body weight. After 72 hr of LPS administration, young mice were intra-venously injected with 10^6 Group A GMSCs. Similarly, adult and elderly mice were administered intra-venously with Group B and Group C GMSCs respectively. The experiments were conducted in triplicate with three individual samples from each group of GMSCs (4 animals per sample, a total of 12 animals for each age group of GMSCs). The experiment was terminated after 96 hr of GMSC administration, by sacrificing the experimental animals and analysing their lung tissues as described earlier section (Fig. S2 and Table S1).

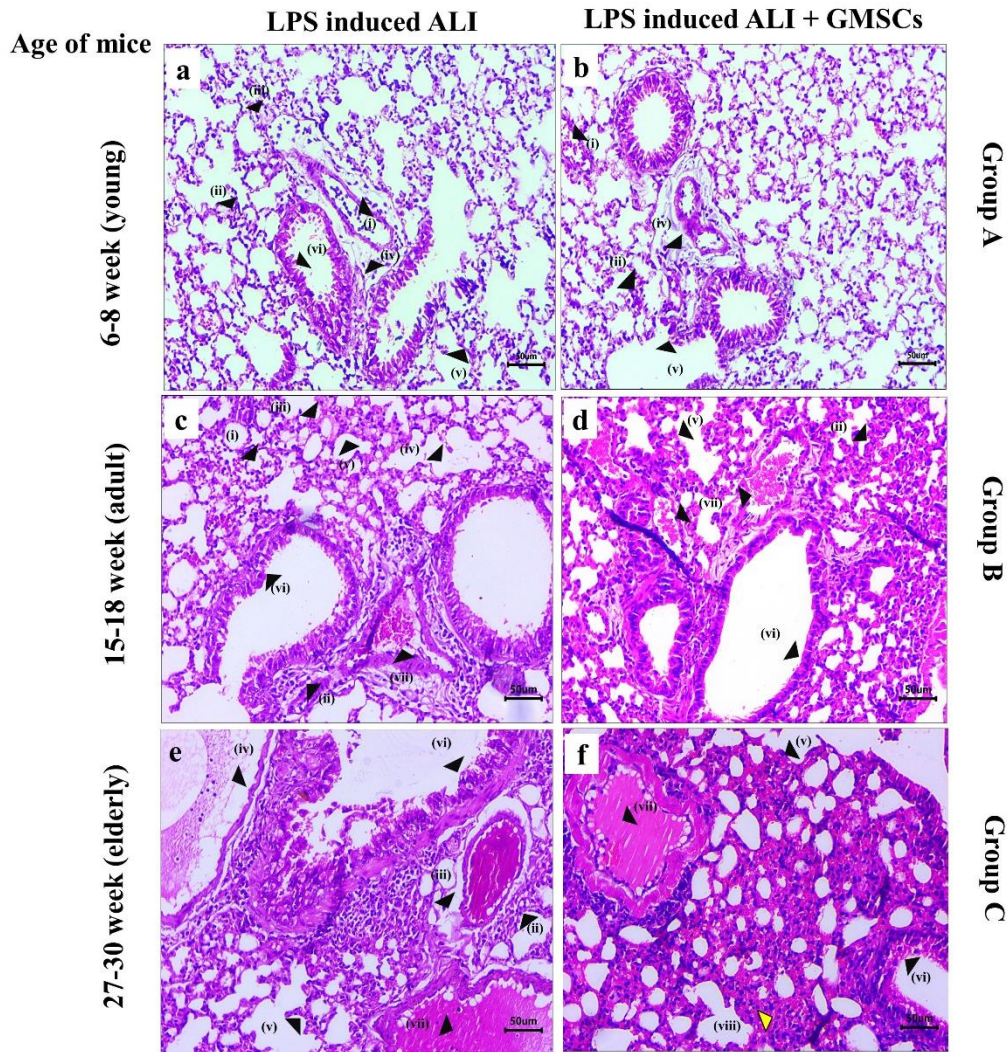


Fig. S2. Histological analysis of lungs with/without GMSCs administration: (a-f) Histological analysis of LPS induced ALI in young (6-8 week), adult (15-18 week) and elderly (27-30 week) Swiss albino mice, showed maximum severity of lung damage in elderly mice, as represented by large foci of thrombus and accumulation of proteinaceous debris. The mice from varied age groups displayed different parameters of lung injury after 7 days of LPS administration and administration of GMSCs was observed to alleviate those particular parameters. For instance, (b) Group A GMSCs reduced the number of infiltrated neutrophils in the alveolar and interstitial space; lowered the sites of hyaline membrane and proteinaceous debris and also improved the thickness of alveolar septa in young mice. On the other hand, (c and e) both adult and elderly mice respectively, displayed lower infiltration of neutrophils but

higher instances of proteinaceous debris, hyaline membrane, septal thickening, thrombus formation and intra-alveolar haemorrhages. (d and f) administration of Group B and Group C GMSCs in these mice respectively has been observed to reduce the severity of lung injury with respect to most of the parameters (Magnification 20x; scale 50 μ m). Histological parameters studied: (i) infiltration of neutrophils in alveoli and (ii) interstitial spaces; (iii) hyaline membrane formed due to fibrin polymerization that leaks into the interstitial/ alveolar space; (iv) presence of proteinaceous debris in the alveolar space (such as fibrin strands); (v) thickening of alveolar septa; (vi) desquamation of alveolar epithelium; (vii) thrombosis; (viii) intra-alveolar haemorrhages.

Table S1.

	Age of Mice	6-8 week		15-18 week		27-30 week	
	Groups Criteria	LPS	LPS + Group A	LPS	LPS + Group B	LPS	LPS + Group C
(i)	Neutrophils in alveolar space	+++++	+++	++++	+	++++	+
(ii)	Neutrophils on interstitial space	+++++	+++	+++++	+++	+++++	+++
(iii)	Hyaline membrane	+++	-	++++	++	+++++	+
(iv)	Proteinaceous debris	++	+	+++	++	++++	++
(v)	Thickening of alveolar septa	3x	2x	3.5x	2x	3.5x	3x
(vi)	Desquamation of alveolar epithelial cells	++	+	++++	+++	+++++	++++
(vii)	Thrombosis	+++	+	++++ (large foci)	+++ (small foci)	+++++ (very large foci)	+++ (very small foci)
(viii)	Intra-alveolar hemorrhage	-	-	-	+++	-	+++

Table S1: Comparative analysis of structural changes in LPS induced ALI with/ without GMSCs administration. Scoring of histological sections of LPS induced ALI in young (6-8 week), adult (15-18 week) and elderly (27-30 week) Swiss albino mice. The mice from varied

age groups displayed different parameters of lung injury after 7 days of LPS administration and administration of GMSCs was observed to alleviate those particular parameters. (“+” represents presence of a given parameter in at least 10 locations of a histological section. 8 such sections from different mice of each group were analysed to score the severity of lung damage. All the histological parameters were compared with sham control).

Table S2. List of primers

	Gene Name	Forward Primer	Reverse Primer
	Human primers		
1.	FGFR	AAACCGTATGCCCCGTAGCTC	AGGTGGCATAACGGACCTTG
2.	PDGFR	ATGCTCAGCAGAGTGTCATCC	CCACTTCTTTGCGGGGGTA
3.	EGFR	TATTGATCGGGAGAGCCGGA	TCGTGCCTTGGCAAACCTTC
4.	CDKN1A	CCAGCATGACAGATTTCTACCAC	AGAAGATGTAGAGCGGGCCT
5.	CDKN2A	CTTGGTGACCCTCCGGATTC	TCATCATGACCTGGTCTTCTAGG
6.	p53	CCTATGGAAACTACTTCCTGAAAAC	CTGGCATTCTGGGAGCTTCA
7.	SIRT1	TTGCCGGAAACAATACCTCC	CCACATGAAACAGACACCCCA
8.	GAPDH	TCGGAGTCAACGGATTTGGT	TCGCCCCACTTGATTTTGA
	Mouse primers		
9.	TNFα	GTAGCCCACGTCGTAGCAAA	ACAAGGTACAACCCATCGGC
10.	TGFβ	CTGCTGACCCCCACTGATAC	GGGGCTGATCCCGTTGATT
11.	IFNγ	AGACAATCAGGCCATCAGCAA	TGTGGGTTGTTGACCTCAAACCT
12.	GAPDH	CAGGAGAGTGTTTCCTCGTCC	TTTGCCGTGAGTGGAGTCAT

Table S3. List of products with catalogue numbers

Sr. No.	Product	Make	Catalogue
1.	Alpha Minimal Essential Media (α MEM)	Sigma Aldrich	M8042
2.	Foetal Bovine Serum (FBS)	Gibco	16000044
3.	Trypsin Phosphate Versene Glucose (TPVG)	HiMedia	TCL031
4.	Trypan Blue	HiMedia	TC193
5.	Paraformaldehyde	HiMedia	TCL119
6.	Eosin	HiMedia	TC357
7.	MTT	Himedia	MB186
8.	Anti-human CD44	BD Bioscience	560977
9.	Anti-human CD90	BD Bioscience	560977
10.	Anti-human CD73	eBioscience	12073942
11.	Anti-human CD105	eBioscience	12105742
12.	Anti-human CD34	BD Bioscience	560941
13.	Anti-human CD45	BD Bioscience	560974
14.	X-Gal	HiMedia	MB069
15.	GoTaq qPCR Master Mix	Promega	A6001
16.	iScript cDNA Synthesis Kit	BioRad	1708890
17.	Adipogenic Differentiation Kit	Lonza	PT3004
18.	Laminin	Invitrogen	23017015
19.	Poly-D-Lysine	MP Biomedical	150175

20.	DMEM-F12	Gibco	11320033
21.	Fibroblast Growth Factor 2 (FGF2)	Gibco	PHG0024
22.	Epidermal Growth Factor (EGF)	Gibco	PHG0314
23.	N2 Supplement	Gibco	17502048
24.	Anti-Human Nestin conjugated with Alexa fluor 555	Invitrogen	MA1-110-A555
25.	Anti-Human β III Tubulin	Invitrogen	MA1-19187
26.	Goat Anti-mouse FITC	Invitrogen	F-2761
27.	Ultra Cruz Mounting Medium	Santa Cruz Biotechnology	sc-24941
28.	StemPro Osteogenic Differentiation Kit	Gibco	A10072-01
29.	Alizarin Red S	Sigma Aldrich	A5533
30.	Para-nitro phenol phosphate (pNPP)	SRL	144816
31.	AMP	Sigma Aldrich	A9199
32.	Para-nitro phenol (pNP)	Sigma Aldrich	N7660
33.	Anti-Human Osteocalcin	Abcam	ab93876
34.	Anti-Human Collagen Type I	Abcam	ab34710
35.	Donkey Anti-rabbit FITC	Santa Cruz Biotechnology	sc-2090
36.	Phytohemagglutinin (PHA)	Sigma Aldrich	11082132001
37.	Histopaque	Sigma Aldrich	10771
38.	Lipopolysaccharide	Sigma Aldrich	L8274
39.	PowerUp SYBR Green Master Mix	Applied Biosystems	A25742
40.	Verso cDNA Synthesis Kit	Invitrogen	ab1453A

41.	Formaldehyde	HiMedia	AS127
42.	Rhodamine Phalloidin	Invitrogen	R415
43.	Phalloidin FITC	Sigma Aldrich	P5282
44.	DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride)	Invitrogen	D1306
45.	Oil Red O	Sigma Aldrich	O0625

Video. S1. Time dependent *in vitro* migration of GMSCs for wound healing.