IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 6 Ver. 14 (June. 2018), PP 32-40
www.iosrjournals.org

A comparative study on immunomodulatory potential of tissue specific hMSCs: Role of HLA-G

Sonali Rawat[1], Pallavi Srivastava[1], Punit Prabha[1], Suchi Gupta [1], Uma Kanga[2*], Sujata Mohanty[1*]

[1]*Stem Cell Facility, DBT-Centre for Excellence of Stem Cell Research, AIIMS, Ansari Nagar, New Delhi-29*
[2]*ICMR-Centre for Excellence in Molecular Medicine, Department of Transplant Immunology and Immunogenetics, AIIMS, Ansari Nagar, New Delhi-29*
Corresponding author:Sujata Mohanty[1](drmohantysujata@gmail.com), Uma Kanga[2](umakanga@hotmail.com)

Abstract:*Human Mesenchymal Stem Cells (hMSCs) are able to modulate the immune response by altering the proliferation and function of various types of immune cells. hMSCs tends to secrete various paracrine factors including immunomodulatory factors such as IDO, PGE-2, TGF-B1, HLA-G IL-10 etc. Among these, HLA-G is aunique molecule because of its property to show immune responses in both forms, soluble and surface expression.*

In present study, we have explored the immunomodulatory property of hMSCs derived from various tissues sources {(bone marrow (BM), adipose (AD) & dental pulp (DP)} by evaluating HLA-G expression at transcriptional and translational by qRT-PCR, Immunofluorescence, flow cytometry and ELISA assay. To evaluate the immunosuppressive property of tissue specific hMSCs, they were co-cultured with allogeneic peripheral blood mononuclear cells (PBMNCs) and were assessed by proliferation of PBMNCs.Transcriptional studies showed that expression level of panHLA-G was similar in all tissue specific hMSCs. Cytoplasmic and Surface protein expression study revealed that intracytoplasmic expression of HLA-G1/G5 in hMSCs was significantly higher than the surface expression of HLA-G1 derived from various tissue sources. Functional immunosuppression studies demonstrated that immune suppression caused by DP-MSCs and AD-MSCs was highest among all the three tissue sources. Overall finding suggests that all cell types showed comparable immunomodulatory effect. However, immunosuppressive ability was maximal in the case of DP-MSCs followed by AD-MSCs and BM-MSCs.These tissue specific hMSCs were able to hamper the PBMNCs mediated immune response by inhibiting their proliferation under the allogenic experimental condition.Furthermore, despite the similarities in immunomodulatory properties of hMSCs from three tissues sources, these results indicate the potential use of i.e., DP-MSCs and AD-MSCs, for translational purposes.

Keywords:*Mesenchymal Stem Cells; Immunomodulation; Dental pulp derived stem cells; HLA-G; Allogenic*

Date of Submission: 12-06-2018 Date of Acceptance: 27-06-2018

I. Introduction

Human Mesenchymal Stem Cells (hMSCs)originate from mesenchyme tissue. They are also known as multipotent stromal cells. hMSCs are non-hematopoietic progenitor cells which primarily isolated from the Bone Marrow. Though now they have been successfully isolated from other tissues as well including Adipose(AD), Dental Pulp (DP), Umbilical cord(UC), Cord blood etc[1,2]. These cells are subjected to global interest owing to their immuno-modulatory capacity along with multi-lineage differentiation potential and lack of ethical concerns. The discovery of peripheral tolerance induced by hMSCs was a breakthrough and thenitwas suggested for the first time that hMSCs could be used as therapeutic tools against immune-mediated disorders and Graft versus Host Disease(GvHD) [3].

They are mainly characterized by surface marker expression of CD73, CD90, CD54, CD44 and CD105 markers. They are even characterized by the absence of Hematopoietic markers CD11b, CD34, CD19, CD45 and HLA-DR [4,5]. Other important characteristic of hMSCs is their immunomodulatory capacity which refers to their capacity to interfere with differentiation and functions of multiple immune cells [6]. In *in-vitro*, hMSCs have been observed to suppress the proliferation of T cells upon stimulation; inhibit monocyte-derived DC for differentiation and maturation, proliferation of B cells and natural killer cells, also promote the generation of regulatory T cells [7,8]. hMSCs owe these characteristics to several soluble and membrane bound immunomodulatory molecules such as IDO (Indoleamine2,3-dioxygenase), IL-10, Prostaglandin E2 (PGE-2), Nitric Oxide(NO) and Human Leukocyte Antigen-G (HLA-G1/G5) [9,10]. These characteristics support the possibility of exploiting universal donor hMSCs for therapeutic applications.

The first report of HLA-G expression was in trophoblast cells which are known for protection of maternal NK cell mediated cytolysis. This study identified that HLA-G was a key molecule in fetal-maternal tolerance [11]. on further exploration, it became evident that HLA- G plays an important role not only in fetal-maternal tolerance, but also in regulating immune responses, promoting and maintaining peripheral tolerance in pathological conditions. It can act on diverse types of immune cells and regulate short and long-term immune-responses.It was reportedthat immunomodulatory potential may differ among the different tissue specifichMSCs, which gives us a clue to evaluate the expression level of HLA-G in tissue specific sources [10].

Therefore, in present study we aimed to compare the biological characteristics of hMSCs originating from different tissues, i.e. BM-MSCs, AD-MSCs and DP-MSCs, with respect to immunomodulatory capacity. The characterization of hMSCs derived from different tissues with identifying molecular signatures may prove to be helpful for selecting a suitable source for a specified clinical application.

II. Material and Methods

2.1 Isolation and culture of hMSCs from BM, AD and DP: The study was approved by Institutional committee for Stem Cell Research (ICSCR) (Letter no.ICSCR/54/16CR). All the samples were obtained after taking donor's consent.

2.1.1 Bone Marrow derived Mesenchymal Stem Cells (BM-MSCs)

Bone Marrow was collected from the donor (n=5) undergoing the routine medical test procedure at AIIMS, New Delhi. hMSCs were isolated and cultured as previously described. Briefly, neat bone marrow was seeded in 60mm culture dish (BD, USA) in complete growth media containing 1X Dulbecco's Modified Eagle Medium – Low Glucose (DMEM-LG) (Life Technologies, USA) media with 10% Fetal Bovine Serum (FBS) (HyClone, USA), 1%Penicillin (100U/ml) + Streptomycin (100ug/ml) [Life Technologies, USA]. The cells were incubated in a humidified atmosphere at 37°C with 5% CO_2. Medium was changed every third day until the cell confluency reached to 80%. Adherent cells were then passaged with 0.05% trypsin-EDTA (Invitrogen-Gibco) and reseeded at $1X10^4$ cell/cm^2. [12,13]

2.1.2 Adipose Tissue derived Mesenchymal Stem Cells (AD-MSCs)

Adipose tissue was collected from donor (n=5) undergoing pre-scheduled surgical procedure at AIIMS, New Delhi. The sample was collected in a 5ml transport vial containing DMEM-LG without FBS with 1% penicillin (100U/ml), Streptomycin (100ug/ml) + Gentamycin(250ug/ml). The Samples were washedwith 1X of PBS containing 1%Penicillin (100U/ml) + Streptomycin (100ug/ml) + Gentamycin(250ug/ml). Then explant (~2mm) was carefully placed in a 35mm culture dish and kept undisturbed. Incubated at 37˚C and 5%CO_2 for overnight. Next day, as the tissue adhered to the surface, complete media was added and changed every three to four days. When cells started growing and migrating out of the explant and they reached 80% confluence, cells were harvested using 0.05% trypsin-EDTA (Invitrogen-Gibco) and transferred into a 60mm culture dish for further experiments.

2.1.3 Dental Pulp derived Mesenchymal Stem Cells (DP-MSCs)

Third molars were obtained from each individual (n=5) for orthodontic treatment at AIIMS, New Delhi.For DP-MSCs isolation, tooth was rinsed with 1XPBS containing antibiotics (1% penicillin (100U/ml), Streptomycin (100ug/ml) + Gentamycin (250ug/ml) and dissected with bone cutter to extract the Dental pulp section. Pulp was then chopped into the fine pieces and explant(2mm) for the same was plated on to the 35mm petri plate. Next day, as tissue adhered to the surface and for further maintenance of the tissue they were maintained at 37°C, 5% CO_2, in DMEM-Low glucose 10% FBS and the media was changed every three to four days. When explant cells started growing and migrating out of the explant and when cells reach 80% confluence, they were harvested using 0.05% trypsin-EDTA and plated into a new 60mm culture dish [14].

Culture were monitored by phase contrast microscopy (Olympus, Central Vally, Japan) in order to evaluate the cell morphology and confluency. All assays were performed using hMSCs at passage 3, after their immunophenotypic analysis.

2.1.4 Trilineage differentiation

The technique was performed as per the previous established laboratory protocol [12,13,14].

2.1.5 Population doubling time (PDT)

hMSCs for each sample (N=3) were seeded at a density of$50x10^3$ cells per35mm petri dish (Becton Dickinson, USA). After 72hrs, hMSCs were enumerated and assessed for viability using Trypan Blue dye exclusion assay. The PDT was obtained by the formula [14].

PDT=T−To Log2 (LogN−LogNo)

Where, T: Time of harvesting

To: Time of seeding

N: Number of cells harvested

No: Number of cells seeded

2.1.6 Measurement of Metabolic Activity by MTT assay
Proliferation rate of hMSCs (N=3) was performed at Day 1,3,5,7 and 14 and were measured by 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The technique was performed as per the previous established protocol [14]

2.2 Immunophenotyping
At passage 3(P3), cells were characterized using monoclonal antibodies specific for CD105-APC, CD73-PE, CD29-FITC, CD90-PerCp-Cy5.5, HLA-ABC-APC, HLA-DR-FITC, CD34/45-PE/FITC (BD Pharmingen, France). Acquisition and data analysis were performed using flow cytometry BD-LSR-II (BD Bioscience) and FACS Diva Software Version 6.2. For, HLA-G Flow cytometry studies, we used mouse anti HLA-G1/HLA-G5 MEMG/9 PE antibody (Exbio, Praha, Czech Republic) at 1/200 final concentration for 75000 cells and incubated for 40mins at 4°C in dark. For analysis isotype controls were included. Intracellular staining was performed using 0.05%Tween-20 in 2% BSA for 20 mins at 4°C followed by washing with PBS.
To detect soluble HLA-G molecule in culture supernatant, ELISA coated with MEMG/9 antibody was performed according to the manufacturer's instruction (Exbio, Praha, Czech Republic).

2.3 Quantitative Real-time Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR)
For the isolation of total RNA from cultured hMSCs cells, the respective T-25 flasks were washed using PBS for the removal of any existing debris or serum. The cells were then trypsinized using Trypsin-EDTA, mixed with fresh media, and pellet down at 1200 rpm (Beckman Coulter, California, USA) for 10 minutes. The cells were transferred in to micro centrifuge tubes (MCT) and were then lysed using TRI reagent (Molecular Research Centre, Ohio, USA), 1ml/ 1x 10^6 cells. The total RNA was prepared according to the phenol chloroform method. The concentration and OD of samples were recorded using a Nanophotometer (Implen, Germany).

Reverse Transcriptase PCR: cDNA was prepared, using 5µg of the RNA samples from tissue specific hMSCs and JEG-3 by Reverse transcriptase (RT) enzyme (Promega, USA). **Optimization of cDNA using GAPDH:** Glyceraldehyde – 3 phosphate dehydrogenase (GAPDH) was used in the PCR setup as the housekeeping gene, for the optimization of the prepared cDNA samples of hMSCs and JEG-3. qPCR was performed in duplicated using SYBR green Master Mix according to the manufacturer's instruction (Kappa). **Primers** for GAPDH:
Forward Primer: 5' – GAG TCA ACG GAT TTG GTC GT – 3' **Reverse Primer:** 5'– GAC AAG CTT CCC GTT CTC AG – 3'. **HLA-G: Forward Primer**: 5'-CTGACCGAGACCTGGGCGGGCT-3 '**ReversePrimer:5'-**GGCTCCATCCTCGGACACGCCGA-3' We calculated the average of panHLA-G value from 5 healthy donors of tissue specific hMSCs in duplicate using equation of standard curve.

2.4 Immunofluorescence
Immunofluorescence studies were performed for the detection of intracellular and membrane bound HLA-G protein levels in the hMSCs samples as well as in the positive control JEG-3 cells. Trypsinized cells were seeded on cover slips kept in a 35mm dish (BD Biosciences, USA), hMSCs expansion media were added and incubated at 37°C, 5% CO_2 once confluency reached within 40-50%, the existing media from the dish was removed and cells were washed with PBS. Cells were fixed with 1ml of 4% Paraformaldehyde (PFA) for 20 mins at 4°C. Permeabilization and blocking were done with 0.05% Tween-20 followed by 2% BSA in PBS for 30 mins at room temperature. hMSCs and JEG-3 were incubated with the Mouse monoclonal Anti 4H84 (Abcam, Cambridge, USA) primary antibody (1/150) for overnight at 4°C. Next day, after washing with PBS, cells were incubated with the secondary fluorescent antibody (1/500) for 40 mins at room temperature. To visualize nuclei, slides were stained with dilution 1/4000 DAPI for 3 mins followed by thorough washing of the cells. The acquisition and imaging of the cells were performed using Confocal Microscopy (Leica).

2.5 Peripheral Blood Mononuclear Cells (PBMNCs) isolation and one way Mixed Lymphocyte Reaction (MLR)
Isolation of Peripheral Blood Mononuclear Cells (PBMNCs): Ficollhypaque method was usedfor isolation of PBMNCs, Buffy layer was collected and washed twice using PBS. The final pellet was resuspended in 1ml of Complete Rosewell Park Memorial Institute – 1640 media (RPMI-1640 + 10% FBS, 10mM Penicillin Streptomycin, 10mM L- glutamine), and cells were counted using the Neubauer's chamber.

Mixed Lymphocyte Reaction – Culture Setup: In a 96flat bottom well plate, hMSCs: PBMNCs were harvested in the ratio of 1:5. Therefore to 5x 10^4 cells per hMSCs samples in each well, 2.5 x 10^5 cells of PBMNCs were added along with RPMI-1640 media. A mitogen – Phytohaemagglutinin (PHA) was added to the PBMNCs at a concentration of 10µg/ml(Sigma), so that the PBMNC proliferation could be activated. Negative control co-culture with no PHA added was also put up. Only PBMNCs activated with PHA were used as

positive control for comparison with the PBMNCs which were plated in close contact with hMSCs samples. Experiment was completed in triplicates. The culture plate was incubated in 37°C, 5% CO_2 and plate was observed for 4 days. On day 4, MTT assay was performed to check the proliferation rate of PBMNCs.

Statistical analysis

In this study, all the values have been stated as means (Standard deviation). The statistical test – Ordinary One-Way ANOVA with Tukey Post-hoc test was used for the assessment of significance of the result data obtained. The level of significance was set at $p \leq 0.05$. The analysis of all data and statistical significance was done using GraphPad Prism 5 software.

III. Result

1.Characterization of human adult BM-MSCs, AD-MSCs and DP-MSCs

1.1Morphological Analysis: It is known that hMSCs from various sources exhibit similar morphological characteristics, only varying in some intricate details. All tissue specific MSCs revealed flattened spindle shaped cellswith fibroblast like appearance,cells were often interconnected by adhesion structures located on the cell body and on cytoplasmic processes contacting other cells. These characteristics were observed to be well preserved during repeated subculture (Fig 1A).

1.2 Growth Kinetics: To evaluate the difference in growth kinetics tissues specific hMSCs(n=3) were assessed for theirproliferation from day 1 to day14 by MTT assay. All three cells sources exhibited an exponential growth till 1-5days out of which DP-MSCs showed the highest proliferation followed by AD-MSCs and BM-MSCs, thereafter all three sources showed declined growth rate. (Fig 1B). The PDT of DP-MSCs, AD-MSCs and BM-MSCs for P3 cells was 25.28 ±1.91,26.03±2.10 and33.68±1.51hrs, respectively (Fig 1C). DP-MSCs and AD-MSCs grew rapidly and had lowest PDT whereas it was highest for BM-MSCs.

1.3 Surface marker profiling by Flow cytometry: Tissue specific hMSCs, BM-MSCs, AD-MSCs and DP-MSCs at passage 3 were subjected to phenotypic characterization. Flow cytometric evaluation revealed positivity for CD105, CD73, CD90, CD29 and HLA-ABC molecules whereas HLA-DR and CD34/45 a hematopoietic marker were absent on these as detected by flow cytometry analysis (Fig 1.1A).

1.4 Tri-lineage differentiation: Tissue specific hMSCs showed the successful differentiation into osteocytes, chondrocytes and adipocytes. (Fig 1.1B)

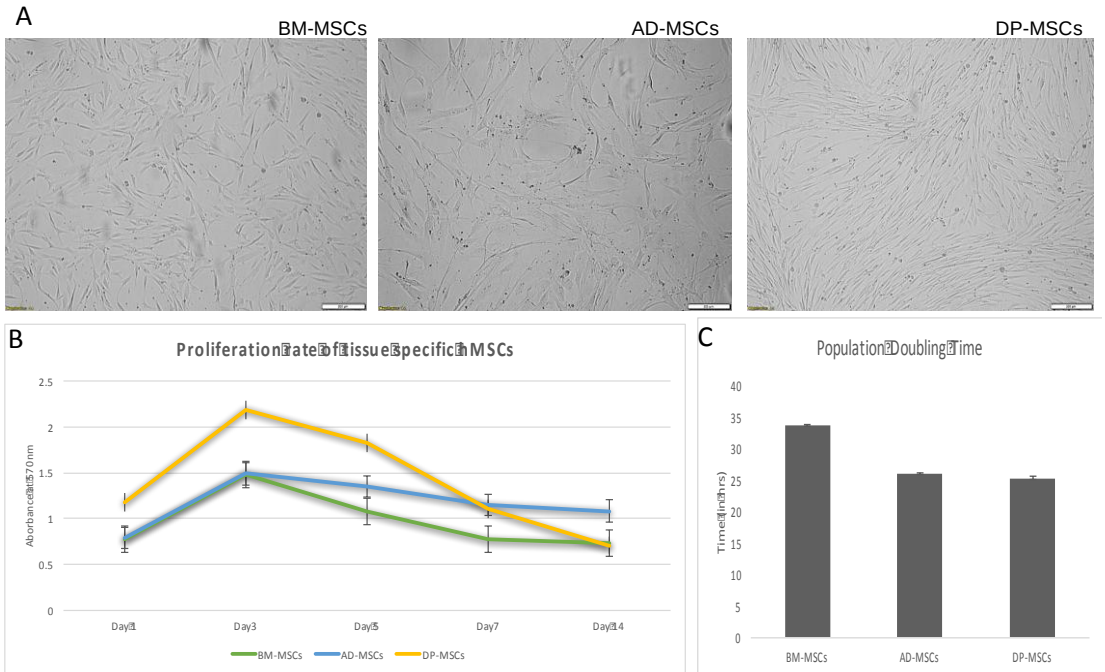

Fig 1: Characterization of tissue specifichMSCs (BM-MSCs, AD-MSCs and DP-MSCs. (A) Bright field images of cells in monolayer depicting the spindle shaped morphology of the isolated hMSCs. (B) Growth kinetics of tissue specific hMSCs. Line curve showing proliferation rate of tissue specific hMSCs from day 1 to 14 days using MTT assay. (C) Bar Graph depicting PDT of BM-MSCs is higher in comparison to AD-MSCs and DP-MSCs

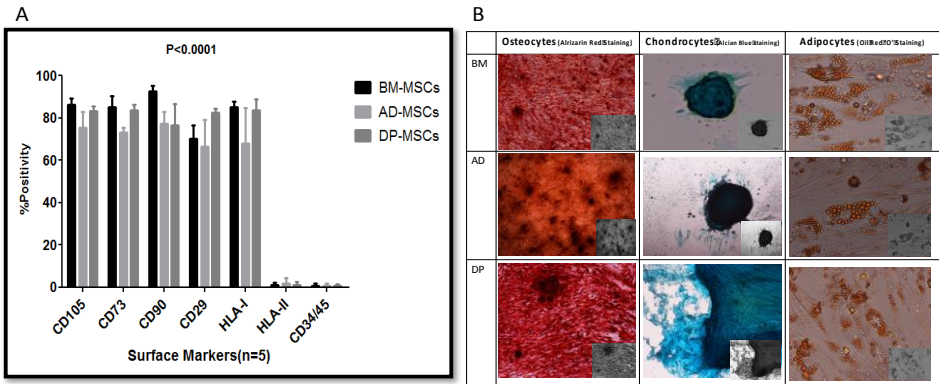

Fig 1.1 (A) Graph depicting the expression level of surface markers in all three types of hMSCs, by flow cytometry. (B) Image showing the successful differentiation of hMSCs into osteocytes (Alizarin Red Staining), chondrocytes (Alcian Blue Staining) and adipocytes (Oil Red O Staining).

2. Expression of the panHLA-G molecule in tissue specific hMSCs at transcriptional level

Quantitative Real Time-PCR was done to analyse the expression of panHLA-G transcript in BM-MSCs, AD-MSCs and DP-MSCs. Graph is plotted on the basis of relative expression of panHLA-G (delta delta CT values), which means higher the CT value, lower the expression of gene of interest(HLA-G). As shown in Fig 2, tissue specific hMSCs expressed varied level of panHLA-G i.e., BM-MSCs :8.354±0.52, AD-MSCs: 3.548±0.66 and DP-MSCs :3.954±0.81. AD-MSCs and DP-MSCs showed the maximum amount of panHLA-G transcript whereas BM-MSCs showed least expression. The JEG-3 cell line was used as a positive control (data not shown) and is known to express a high level of panHLA-G mRNA [15].

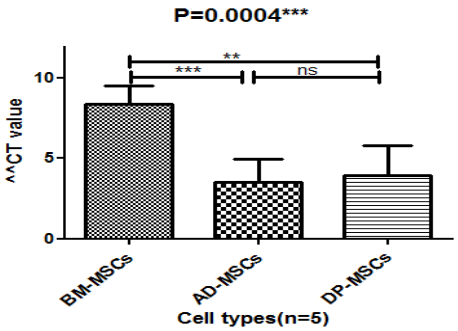

Fig 2: Expression of panHLA-G mRNA by tissue specific hMSCs. Δ Δ CT value of the samples were calculated by keeping JEG-3 cell line as a positive control.

3. Expression of the HLA-G molecule in tissue specific MSCs at translational level

HLA-G can be expressed as seven distinct protein isoforms, alternatively spliced transcript, out of these seven isoforms hMSCs were able to expression surface bound (G1) and soluble (G5).To understand the expression level of different isoforms, we performed surface and intracellular immunofluorescence and flow cytometry by using 4H84 and MEM-G/9 respectively. As shown in Fig 3; discrete staining of HLA-G indicated higher expression at intracellular level than surface which is readily detectable on all tissue specific hMSCs and JEG-3; (a positive control). The basal level of expression was quantified in all tissue specific hMSCs by ELISA as HLA-G5 exists as a soluble isoform. Results showed that supernatant from 80-90% confluent monolayer of BM-MSCs, AD-MSCs and DP-MSCs, obtained after 48hrs of culture contained soluble HLA-G5 protein at a concentration of 0.053±0.0001U/ml in BM-MSCs, 0.139±0.005U/ml in AD-MSCs, 0.2414±0.005U/ml in DP-MSCs, 11.143±0.051U/ml in JEG-3 and 0.009±0.014U/ml LCL (negative control), DP-MSCs showed significantly high expression of soluble HLA-G, with respect to other tissue specific hMSCs (Fig4A). Presence of HLA-G1/G5 in tissue specific hMSCs was also quantified by flow cytometry using the specific MEM-G/9-PE antibody on 5 different healthy donors from each tissue specific sources. Surface and intracellular staining was used to study the membrane-bound and intracytoplasmic HLA-G protein respectively. As shown in Fig 4B(i); surface expression of HLA-G was observed in 4.66±0.7% of BM-MSCs, 8.940±0.63% of AD-MSCs and 8.780±1.081% of DP-MSCs Intracellular expression of HLA-G was observed in 35.52±3.820% of BM-MSCs, 46.02±4.53% of AD-MSCs and 27.40±3.696% of DP-MSCs (Fig 4B(ii)). These results signify the highest expression of HLA-G is exhibited in AD-MSCs followed by DP-MSCs and least expression in BM-MSCs.

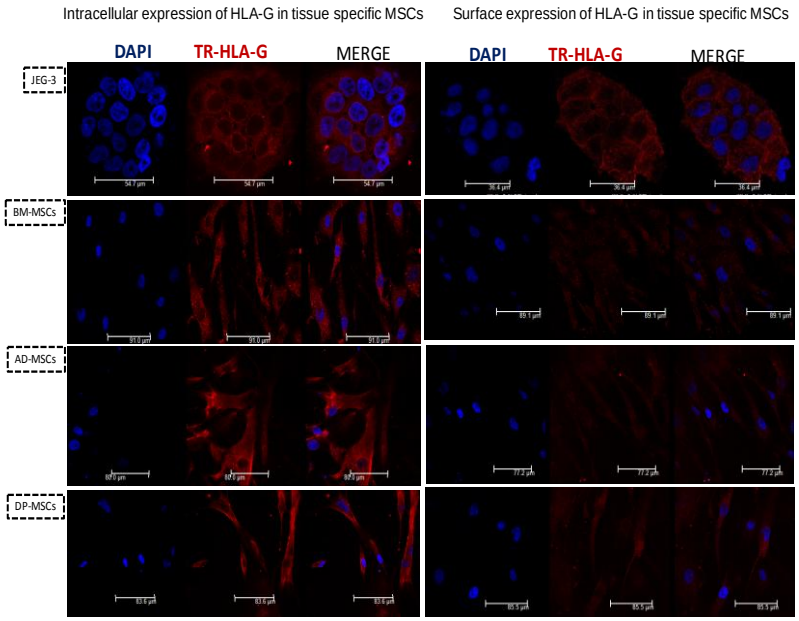

Fig 3: Immunofluorescence images of tissue specific hMSCs. The figure shows the presence of HLA-G in the cytoplasmic and surface region of hMSCs. This confirms that the HLA-G protein is highly expressed in the cytoplasmic region of the hMSCs when compared to surface region.

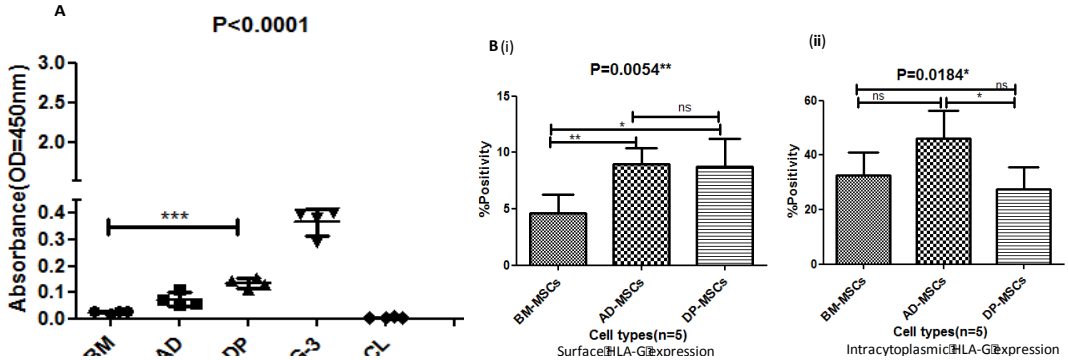

Fig 4: (A). Basal level expression of sHLA-G(-G5) in tissue specific hMSCs by ELISA(n=4). (B) Flow cytometric analysis (i) Surface expression level of HLA-G-1 in all three tissue types and (ii) Intracellular expression level of HLA-G-5 in hMSCs from all three tissue types.

4. Level of immunosuppression by tissue specific hMSCs in one way MLR

The Immuno-suppressive capacity of these hMSCs was assessed and compared using mixed lymphocyte reaction – co-culture experiment. Fig 5 represents the graph plot for MTT data analysis for the MLR experiments using tissue specific hMSCs (n=3).

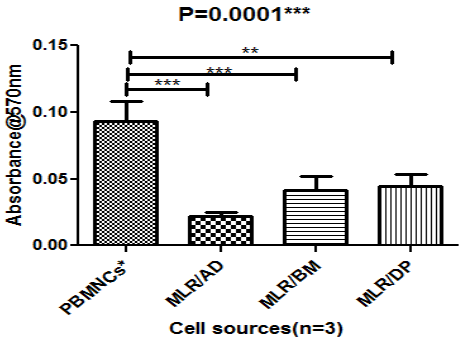

Fig 5: The suppression of proliferation of immune cells (PBMNCs) observed in each case, with respect to the positive control of only PHA activated PBMNCs. P=0.001***

The data were found to be significant ($p \leq 0.05$) as compared to PHA treated PBMNCs. The percentage decrease of the proliferation of immune cells under the effect of hMSCs in the co-culture with respect to the positive

control is calculated, and therefore the average immune suppressive ability of AD-MSC, BM-MSC and DP-MSC is compared. It is therefore observed that, the immune suppression caused by AD-MSC, BM-MSC and DP-MSC was ~74%, ~63% and ~64% respectively. When relatively compared, the immune suppression caused by AD-MSC was observed to be highest among the three samples, although not by statistically significant difference.

IV. Discussion

Stem cell therapy holds enormous potential for the treatment of various degenerative disorders. Also, it is preferred over conventional therapy because its reparative and regenerative nature. It is often observed that the mostly used cell candidate in such treatments is autologous hMSCs. The reason beingitsavailability and ease of *in vitro* expansion. The hMSCs can be isolated from Bone Marrow, Adipose tissue, Dental Pulp and several other tissues. The growing optimism towards stem cell treatment is based upon the promising outcomes obtained from various studies stating the role of hMSCs in immunomodulation and their translational implications.However, detailed understanding of basic immunomodulatory properties of hMSCs obtained from different tissue sources need to be explored further to ensure their optimum clinical uses. Although the current study focused on the evaluation of expression of the immuno-tolerogenic molecule HLA-G in tissue specific hMSCs.

According to our results, AD-MSCs and DP-MSCs holds great potential to be the alternatives of BM-MSCs for therapeutic purposes because of their ease of collection as compared to the painful bone marrow aspiration. Typical spindle shaped cell morphology was observed in hMSCs, obtained from all the three tissue sources. PDT and metabolic assessment assay showed that DP-MSCs have highest proliferative capacity as compared to those of AD-MSCs and BM-MSCs. Surface marker profiling also revealed similarities in the level of expression CD105, CD90, CD73, CD29, HLA-I, HLA-II and CD34/45. The trilineage capability of these hMSCs showed marginal difference (not quantified) as observed from the staining images. Our results are in line with the already reported data [14,16-18].Further, immunomodulatory property of hMSCs was assessed, in terms of expression level of HLA-G at molecular, cellular and protein levels. Human Choriocarcinoma JEG-3 cell line was taken as the positive control and LCL was negative control. A higher level of HLA-G expression has already been reported in JEG-3 cell line [19]. Relative expression assessment of HLA-G at mRNA level indicated that AD-MSCs and DP-MSCs were atthe comparable expression level; while, BM-MSCs had minimum expression among all the tissue sources.Protein level expression of HLA-G1/G5 was studied by Flow cytometry enumeration, ELISA and immunofluorescence studies. Flow cytometry results revealed higher cellular positivity for intracellular HLA-G in AD-MSCs followed by that in DP-MSCs and BM-MSCs, respectively. Similar trend was observed in case of expression of surface HLA-G among all the tissue specific hMSCs groups. ELISA assay performed on all the tissue specific hMSCs showed that DP-MSCs exhibited highest release of soluble HLA-G5 in the culture supernatant.This resultfurther corroborated the data obtained from flow cytometric enumeration and immunofluorescence. The distinct expression levels of immunoregulatory molecule among the different hMSCs sources clearly highlight the substantial differences in the mechanisms controlling the immunoregulatory roles mediated by tissue specific hMSCs. Our results are in line with the already reported data in cases of BM-MSCs and AD-MSCs. However, to the best of our knowledge, none of the studies till date reported the immunomodulatory property of DP-MSCs in terms of expression level of HLA-G [2,10].To study the functional aspect of immunosuppressive capability of hMSCs, one-way MLR studies were performed by co-culturing hMSCs with allogeneic PBMNCs and analysing the proliferation level of activated PBMNCs under various conditions. Our study revealed that immunosuppression caused by AD-MSCs and DP-MSCs was similar.Whereas, BM-MSCs showed low immunosuppressive capacity among all three groups. The hMSCs secrete various immunomodulatory factors.

Therefore, the differences in immunosuppressive capability of hMSCs from different tissue sources, are likely to be due to variability in expression level of immunomodulatory factors which govern the immunosuppressive activity in the inflamed microenvironment. Owing to the previously reported immune suppressive ability of hMSCs, those studies hadoftenused BM-MSCs and AD-MSCs because they were known to be non-immunogenic [7] and exhibit immunosuppressive effects both *in vitro* and *in vivo*. [8,20,21]. Due to the favourable immunological characteristics of hMSCs, clinical trials have explored their potential in management of GvHD. Le Blanc et al reported the first case of severe acute steroid resistant GvHD successfully treated with hMSCs [3] and otherstudiesalso confirmed the above report as they observed complete remission in some of the patients in the treatment group[7,24].

hMSCs are believed to play important role in the prevention of graft failure and enhancement of graft engraftment. In this regard, studies by Lazarus et al [22], Le Blanc et al [3] and Jiang et al [23] showed positive results. These studies with such encouraging results, are paving the way not only for present ongoing clinical trials in this area but for future as well.Hence, these data support the notion that usage of hMSCs open new insights in the prevention and treatment of graft rejection in tissue and organ transplantation.Also, that AD-MSCs and DP-MSCare most likely to be a promising alternative to BM-MSCs for future clinical applications.

V. Conclusion

Mesenchymal stem cells are known tobe able to modulate the immune response of immune cells. This study shows that despite the similarities in immunomodulatory properties of hMSCs from different tissue sources Dental Pulp-Mesenchymal Stem Cells have superior immunoregulatory property as compared to AD-MSCs and BM-MSCs and thus may prove more optimal source for therapeutic application in autoimmune diseases. Also, current ISCT guidelines does not include immune related functional tests or more detailed molecular validation and it isimportant aspect is to delineate tissue- specific functional differences in hMSCs isolated from different sources.

Acknowledgement

The work has been supported by All India Institute of Medical Sciences, New Delhi and university of grants commission, government of India.

References

[1]. Lee JM, Jung J, Lee HJ et al. Comparison of immunomodulatory effects of placenta mesenchymal stem cells with bone marrow and adipose mesenchymal stem cells. IntImmunopharmacol. 2012 Jun;13(2):21924.

[2]. Sara m. Melief, Willem e. Fibbe, Helene et al. Adipose Tissue-Derived Multipotent Stromal Cells Have a Higher Immunomodulatory Capacity Than Their Bone Marrow-Derived Counterparts. SCTM 2013; 2:455– 463.

[3]. Le Blanc K, Rasmusson I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004; 363: 1439.

[4]. Gebler A., Zabel, O., Seliger B. et al. The immunomodulatory capacity of mesenchymal stem cells. Volume 18, Issue 2, February 2012, Pages 128–134.

[5]. Dominici M., Le Blanc,K., Mueller I. et al. Minimal criteria for defining multipotent mesenchymal stromal cells. ISCT Cytotherapy. 2006;8(4):315-7.

[6]. Hoogduijn M.J., Popp F. et al. The immunomodulatory properties of mesenchymal stem cells and their use for immunotherapy. IntImmunopharmacol. 2010 Dec;10(12):1496-500.

[7]. Le Blanc K., Tammik, C., Rosendahl, K., et al. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. ExpHematol 2003;31(10):890.

[8]. Di Nicola M., Carlo Stella C., Magni M. et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or non specific mitogenic stimuli. Blood 2002;99(10):383.

[9]. Yufangshi, gang zhenghu, juansu et al. Mesenchymal stem cells: a new strategy for immunosuppression and tissue repair. Cell Research 2010;20: 510-518.

[10]. 10.Nasef A, Noelle Mathieu, Alain Chapel et al. Immunosuppression effects of Mesenchymal stem cells: Involvement of HLA-G. Transplantation 2007; 84: 231-237).

[11]. Rouas-Freiss N.,Goncalves R.M., MenierC et al. Direct evidence to support the role of HLA-G in protecting the fetus from maternal uterine natural killer cytolysis. Proc Natl AcadSci U S A 94, 11520-11525.

[12]. Nandy Bose Sushmita, Mohanty S, Singh Manisha, et al. Fibroblast Growth Factor-2 alone as an efficient inducer for differentiation of human bone marrow mesenchymal stem cells into dopaminergic neurons. Journal of Biomedical Science 2014, 21:83.

[13]. Mohanty S, Sushmita Bose Nandy, Jain Gopal Krishan et al. TGF-beta1 contributes to cardiomyogenic like differentiation of human bone marrow mesenchymal stem cells. International Journal of Cardiology 2013 Feb 10;163(1):93-9.

[14]. KakkarAnupama, Sharma Puneet, SankarManimuthu Mani, et al. Effect of Hypoxia on Stemness and Differentiation of Dental Pulp Derived Stem Cells. IOSR-JDMS, Vol15, Issue 8 Ver. VII (August. 2016), PP 102-111.

[15]. Singh Manisha, KakkarAnupama, Sharma Rinkey, et al. Synergistic Effect of BDNF and FGF2 in Efficient Generation of Functional Dopaminergic Neurons from human Mesenchymal Stem Cells. Scientific Reports SREP-17-11209B.

[16]. Moreau P, Mouillot G, Rousseau P et al. HLA-G gene repression is reversed by demethylation. Proc Natl AcadSci U S A 2003; 100:1191–1196.

[17]. M young, W.L., Jeongeun C. et al. Mesenchymal stem cells from cryopreserved human umbilical cord blood. BiochemBiophys Res Commun.Volume 320, Issue 1, 16 July 2004, Pages 273–278

[18]. Naaldijk Y, Staude M, Fedorova V. et al. Effect of different freezing rates during cryopreservation of rat mesenchymal stem cells using combinations of hydroxyethyl starch and dimethylsulfoxide. BMC Biotechnology. August 2012, 12:49

[19]. 19.Bamberger A.M, Jenatschke S, Schulte H.M. et al. Leukemia Inhibitory Factor (LIF) stimulates the Human HLA-G promotor in JEG3 Choriocarcinoma cells. Jr Clinical Endo & Meta. Vol. 85, No. 10 0021-972X/00.

[20]. 20.Maitra B., Szekely E., GjiniK.l. et al. Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation. Bone Marrow Transplant 2004; 33:597–604.

[21]. 21. Polchert D., Sobinsky J., Douglas G. et al. IFN-gamma activation of mesenchymal stem cells for treatment and prevention of graft versus host disease. Eur J Immunol 2008, 38:1745-1755.

[22]. 22.Lazarus H.M., Koc O.N, Devine S.M. et al. Co-transplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. Biol Blood Marrow Transplant 2005; 11:389–398.

[23]. 23.Jiang X.X, Zhang Y., Bing L. et al. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood May 15, 2005 vol. 105 no. 10 4120-4126.

[24]. 24. Suhag H. Parikh, Paul S, Vinod K. Prasad et al. Correction of Chronic Granulomatous Disease After Second Unrelated-Donor Umbilical Cord Blood Transplantation. Pediatr Blood Cancer 2007; 49:982–1046.