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(Research Article)

Immunomodulatory Properties of Human Adipose Mesenchymal Stromal/Stem Cell in Type 2 Diabetes Milieu.

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Abstract: Adipose tissue is a readily available and plentiful source of multipotent mesenchymal stromal/stem cells (AT-MSC). The immunomodulatory properties of AT-MSC are being introduced in type 2 diabetes (T2D) cell- based therapy. The study aimed to uncover the impact of T2D, on the interplay between AT-MSC and immune cells to develop an effective and safe AT-MSC immunotherapeutic modality. Thus, a direct allogenic co-culture of healthy AT-MSC (nAT-MSC) and peripheral blood mononuclear cells (PBMC), from healthy (nPBMC) or T2D (dPBMC) donors, and stimulated with anti-CD3/CD28, was established *in vitro*. PBMC proliferation was evaluated by measuring 5-bromo-20-deoxyuridine (Brdu) incorporation in the DNA of proliferating cells in a colorimetric ELISA assay. Expression levels of $CD3^+$ T cell activation surface markers (CD25 and HLA-DR) were detected using a flow cytometer. As well, the anti-proliferative effect of naïve and interferon gamma (IFN-ɤ) - primed AT-MSC, isolated from T2D patients (dAT-MSC), on autologous PBMC was explored using the Brdu proliferation assay. In the applied co-culture setting, the diabetic milieu does not significantly impact the potential of nAT-MSC to suppress stimulated PBMC proliferation. However, it significantly compromises nAT-MSC ability to modulate the activation markers expression, making them less potent to suppress CD25 and HLA-DR expression. Moreover, the dAT-MSC have attenuated ability to suppress the proliferation of autologous stimulated dPBMC, nevertheless, priming of dAT-MSC with IFN-ɤ, might improve such defect. The results suggest that T2D might affect the immunosuppressive potential of AT-MSC and pre-conditioning of dAT-MSC with a pro-inflammatory stimulus could enhance their therapeutic effect.

Keywords: Type 2 diabetes, Adipose, Mesenchymal, Immunosupressive potential, PBMC, Pro-inflammatory stimuli

1. INTRODUCTION

Type 2 diabetes (T2D) is the predominant form of diabetes mellitus, accounting for nearly 85%–90% of all diabetics[1]. T2D is a multifactorial hyperglycemic state in which imbalanced metabolic and inflammatory pathways are integrated to initiate insulin resistance (IR), compensated by hypersecretion of insulin, leading to exhaustion of β cells[2]. Risk factors of T2D, in addition to genetic predisposition, include sedentary lifestyle factors as excessive dietary intake, physical inactivity, smoking and/or alcohol consumption, environmental factors as pollution, and psychosocial stress factors. All such factors can modulate immune system and activate inflammation[3, 4].

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Adipose tissue is found throughout the adult human body to serve as an energy reservoir and an endocrine organ and is composed of different cell components including mesenchymal stromal/stem cells (AT-MSC) [5]. AT-MSC are expandable fibroblast-like-cell, typically characterized according to the International Society of Cellular Therapy (ISCT) [6], and the International Federation for Adipose Therapeutics and Science (IFATS) [7], by three criteria (1) the potential to adhere to plastic surface, (2) they express a panel of surface protein markers; they are positive for CD90, CD105, CD73 and CD44, while they are negative for CD34, CD45 and HLA-DR, (3) they are able to differentiate into osteogenic, adipogenic and chondrogenic lineages in the presence of the respective proper stimuli.

AT-MSC represent a promising treatment for a wide range of inflammatory and autoimmune diseases[8, 9]as they harbor unique immunomodulatory properties[10, 11]. AT-MSC regulate different immune cells including PBMC. AT-MSC are able to modulate allogenic- or mitogenic- induced activation[12], proliferation [13-15] and functions [12, 14]of PBMC. The immunoregulatory functions of human AT-MSC have been documented in pre-clinical T2D models to improve islet β cell functions and ameliorate IR [16-18]. While few clinical trials have shown improved metabolic indices as glycated Hb [19, 20], many questions remain to be addressed before the establishment of routine AT-MSC treatment for T2D.

Patient-derived (autologous) rather than allogeneic MSC may be the most favorable cell transplantation strategy in clinic, to avoid anti-donor immune responses[21].An interesting study has evaluated the efficacy and safety of autologous bone marrow derived -MSC transplantation in T2D patients to conclude the clinical outcomes as are greatly affected by patient characteristics involving disease duration[22]. Abnormalities of AT-MSC, from T2D patients, in expression of surface and secretory immunomodulatory molecules have been detected favoring a pro-inflammatory phenotype [23]. However, the experimental evidence for the *in vitro* immunosuppressive functions of healthy AT-MSC (nAT-MSC) in T2D environment or AT-MSC, from T2D patients (dAT-MSC) is poor [24, 25].

Uncovering the impact of T2D on AT-MSC immunomodulation would help to establish an effective AT-MSC based therapeutic modality in the inflammatory diseases. In the present study, a direct allogenic co-culture of nAT-MSC and PBMC, isolated from healthy (nPBMC) or T2D (dPBMC) donors and stimulated with anti-CD3/CD28, was established *in vitro*. PBMC proliferation was evaluated by measuring BrdU incorporation in a colorimetric ELISA assay. $CD3^+$ T cell activation surface markers (CD25 and HLA-DR) expression was detected using a flow cytometer. As well, the anti-proliferative effect of naïve and interferon gamma (IFN-ɤ) - primed AT-MSC, isolated from T2D patients (dAT-MSC), on autologous PBMC was explored using the Brdu proliferation assay.

2. METHODS

2.1. Subjects and Ethical Statement

Subjects were patients with T2DM (n=4) and non-diabetic donors (n=6), with age range of 30–65 years and a body mass index ≤ 33. Exclusion criteria were as follows: (a) T1DM or any other specific type of diabetes; (b) acute or chronic complications; (c) cancer; (d) autoimmune disease; (e) infectious disease. The study was approved by the Local Medical Research Ethics Committee of the National Research Centre, Cairo, Egypt (Registration no. 16079)Informed consent was obtained from the study participants.

2.2. AT-MSC Isolation and Culture

Human abdominal subcutaneous adipose tissue samples were obtained during non-acute surgical interventions, such as hernia, cholecystectomy, or surgical liposuction. The obtained AT were processed to isolate AT-MSC as described by **Abu-Shahba *et al***. [23]and **Zuk *et al***. [26]. Adipose tissue sample was extensively washed with Phosphate-Buffer Saline (PBS) (Lonza, Belgium), to remove contaminating debris and excess blood, in a sterile biosafety cabinet (NuAire, USA). AT, were then, minced into small fragments and digested with 1mg/ml human collagenase type 1 (Gibco Life Technologies, USA) at 37 ◦C for 60-90 minutes with gentle agitation.

The collagenase was inactivated by adding an equal volume of low-glucose Dulbecco's modified Eagle's medium (Lonza, USA) supplemented with 10% fetal bovine serum (FBS) (Lonza, Belgium). Subsequently, the samples were centrifuged at 250×g for 15-20 min, then the pellet was suspended in PBS and filtered through a 70-µm nylon cell strainer (Greiner Bio-One, Germany) to remove undigested tissue. The filtrate was centrifuged and the resultant pellet, which represents the stromal vascular fraction, was cultured in a basal culture medium (BM) composed of low-glucose DMEM, 10% FBS, 2% penicillin/streptomycin/amphotericin B (Lonza, USA), and 1% GlutaMAX (Gibco, Life Technologies, USA).

Cells were incubated in a humidified atmosphere containing 5% CO2 at 37 ◦C. Within, 5-

7 days of initial plating, non-adherent cells were removed by BM replacement and the adherent fibroblast-like were maintained in culture until 80-90% cell confluence. The adherent AT-MSC were then detached by Trypsin/EDTA (0.05% trypsin/1.0 mM EDTA (Lonza) and replated at cell density of 2000-4000 cells/cm^2. AT-MSC were expanded for 2-3 passages (P) and media exchange was performed twice a week.

2.3. Peripheral Blood Mononuclear Cell (PBMC) Isolation

About 5–10ml of peripheral blood from healthy (n=10) and T2D (n=10) individuals were collected after informed consent and placed into sterile tubes containing EDTA solution. Mononuclear cells were isolated, as described in the study of **Najar *et al***. [27].Briefly, blood samples were diluted with equal volume PBS and 10 ml of diluted blood sample were layered slowly down overlaid with lymphocyte separation media (density 1,077g/ml, Serana, Germany). The samples were centrifuged 800xg, 30 min, and 25 °C. The buffy coat (PBMC layer) was isolated and washed, PBMC were then frozen in a liquid nitrogen tank till use.

2.4. Immunophenotypic Characterization of AT-MSC

The surface immunophenotype of AT-MSC was assessed with a FACS Canto flow cytometer (BD Biosciences, USA) at P.2/3 according to ISCT [6]. Single staining was done to assess the surface phenotype of AT-MSC. Both nAT-MSC and dAT-MSC were monitored for the expression of the stromal markers; CD90 and CD105, adhesion and migration molecule; CD44, the immunomodulatory molecule, CD73, and the endothelial marker, CD34 and the human leukocyte antigen, HLA-DR. For such purpose, AT-MSC were harvested, washed, counted, and then 50-80 x 10^3 cells were incubated with one of the monoclonal antibodies illustrated in (Table 1) for 15-20 minutes in dark at 4°C. The stained cells were washed with 2% FBS in PBS twice (FACS buffer), then ten thousand cells were analyzed, and unstained cells were used to adjust the flow cytometer "FACS Canto" (BD Biosciences, USA).

2.5. dAT-MSC Priming with IFN-ɤ

To study the effect of IFN-ɤ treatment on dAT-MSC immunosuppressive functions on autologous PBMC, dAT-MSC were primed with IFN-ɤ (100ng/ml) (R&D System) for 48 hr before use, according to **Abu-Shahba *et al***. [23]

2.6. BrdULymphocyte Proliferation Assay

nAT-MSC or dAT-MSC were seeded at 20 x 10^3 cells per well in 96-well flat bottomed plates (Greiner Bio-One) in BM overnight. 10 µg/ml mitomycin C (Sigma–Aldrich, USA, Cat # M4287-2MG) was then added to inhibit AT-MSC proliferation and cells were incubated for 2 h. After extensive washing, a total of 10^5PBMC, suspended in 200µl culture medium/well, were inoculated over the pre-adherent AT-MSC (1:5 AT-MSC: PBMC). Lymphocytes were stimulated by purified antibodies against human CD3 (1ug/ml) (HIT3a, BD Biosciences, Cat # 555336) and human CD28 (0.5ug/ml) (CD28.2, BD Biosciences, 556620). Non-stimulated and stimulated healthy or T2D PBMC mono-culture were conducted as a negative and positive control, respectively. nAT-MSC or dAT-MSC mono-culture were also established. The PBMC or AT-MSC mono-cultures and AT-MSC/PBMC co-cultures were cultivated in RPMI 1640 with 25mM Hepes (Lonza, Cat # BE04-558F), supplemented with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin and 1% GlutaMAX. Lymphocyte proliferation was assessed by cell proliferation ELISA, BrdU colorimetric kit (Roche Applied Science, Germany). 100µm BrdU solution was added to the mono-culture and co-culture at day four for 18h. Lymphocyte proliferation was assessed by measuring BrdU incorporation, according to the manufacturer's instructions, using ELx800 vector (BioTek, USA). The inhibitory percentage of AT-MSC on stimulated PBMC proliferation was estimated as follows: 100-proliferation index. Proliferation index= (absorbance of stimulated PBMC/AT-MSC- abs. AT-MSC/ abs. of stim. PBMC) x 100. The absorbance of blank and background controls were subtracted from the absorbance values of the measured monocultures and co- cultures.

2.7. Flow Cytometer Analysis of PBMC Activation Marker

In this experimental setup, AT-MSC and PBMC co-culture were established in 24-multiple well plate (Greiner Bio-One) with a maintained 1:5 cell ratio (AT-MSC: PBMC). On the 5th day, PBMC from mono-culture and co-culture were harvested, washed and incubated, in dark for 20 minutes at 4°C, with antibodies against CD-45 V450 and CD3- FITC, in addition to, anti-CD25 APC-Cy™7 or HLA-DR APC-R700. Analysis was performed using the FACS Canto flow cytometer. All antibodies from BD Biosciences.

2.8. Quantitative RT-PCR

At day 5, total RNA was isolated from PBMC in mono-culture and co-culture using miRNeasy Mini Kit QIAgen RNA extraction Kit (Qiagen, USA). The RNA concentration was measured using a NanoDrop

device ND-2000 (Thermo Scientific, USA). A total of 1 µg RNA was reverse-transcribed into cDNA using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, USA). Gene expression analysis was performed in duplicate *via* real-time quantitative polymerase chain reaction (qPCR) using an ABI 7500 Fast Real-Time Detection System (Applied Biosystems, CA, USA). TaqMan Gene Expression Assays (Applied Biosystems, USA), listed in (Table 2), were used. The relative mRNA expression level was calculated by the comparative ΔΔ Ct method [28] using GAPDH or 18S as an endogenous control.

Table 1. Antibodies of surface protein markers and manufactures

Antibody	Surface Antigen	Manufacturer
CD34-PeCy7	Hematopoietic progenitor cell antigen	BD Pharmingen
CD44-PE	CD44 antigen (adhesion and migration)	BD Pharmingen
CD45R-V450	RO isoform of leucocyte common antigen	BD Horizon
CD73-APC	Lymphocyte-vascular adhesion protein (L-VAP-2) or Ecto 5'-nucleotidase	BD Pharmingen
CD90-FITC	Thy-1	BD Pharmingen
CD105-PE	Endoglin	BD Pharmingen
HLA-DR-FITC	Major Histocompatibility Complex Class II (MHC II)	BD Pharmingen

Table 2. Gene expression TaqMan probes used in the study

Gene Symbol	**Assay ID**
GADPH	Hs02786624_g1
18S	Hs99999901_s1
IFNG	Hs00989291_m1
TNF-a	Hs00174128_m1
IL4	Hs00174122_m1
IL10	Hs00961622_m1

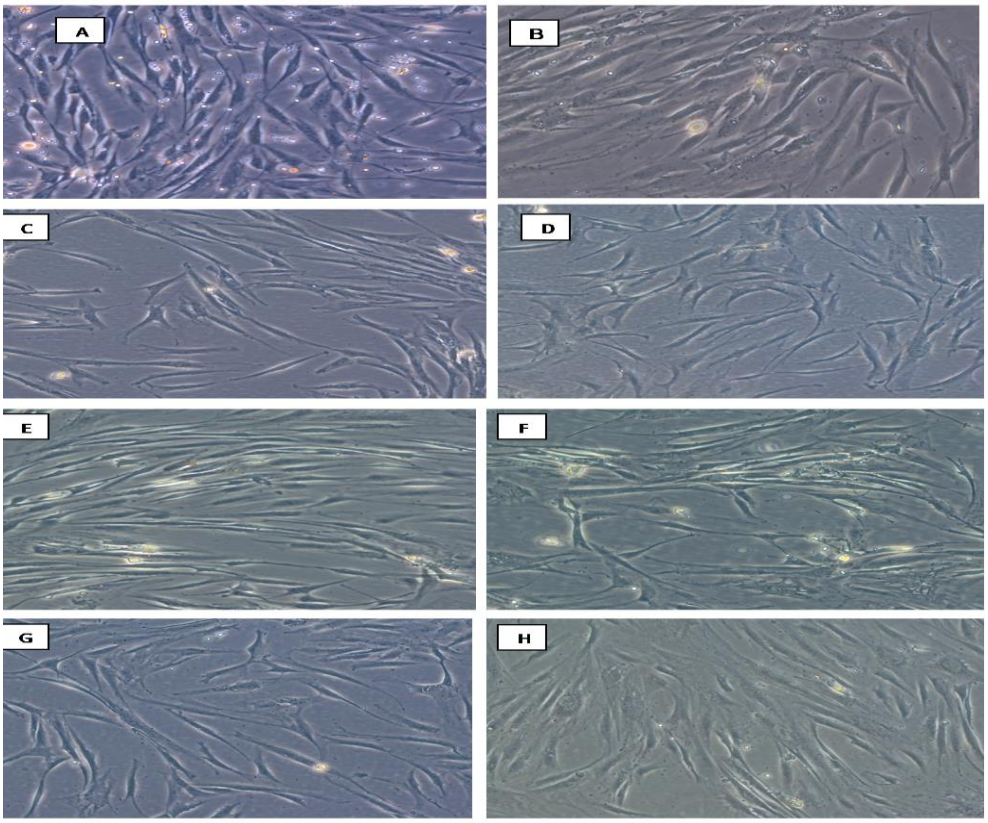

Figure 1. Morphological Characteristics of nAT-MSC and dAT-MSC**.**(A, C, E, G) vs (B, D, F, H) show the typical spindle fibroblast-like morphology of nAT-MSC vs dAT- SC at P0, P1, P2 and P3, respectively. Scale bar = 100 µm.

2.9. Statistical Analysis

Statistical analysis was performed using GraphPad Prism software version 8 (GraphPad Inc.). Data are presented as median with range. The p-values were calculated using a Mann-Whitney test.

3. RESULTS

3.1. Morphological Characteristics

NAT-MSC and dAT-MSC were adherent to the plastic culture surface and they exhibited the typical fibroblast-like-morphology (Fig.1). No striking differences, between nAT-MSC and dAT-MSC, were observed in the microscopic examination for cell morphology during P0-P3.

3.2. AT-MSC Immunophenotypic characterization

Flow cytometer analysis of the surface CD markers revealed that the isolated nAT-MSC and dAT-MSC were positive for CD90, CD44 and CD73 (>85%). However, they were negative for CD34, CD45 and HLA-DR (<5%) (Fig.2).

3.3 Diabetic milieu did not significantly impact AT-MSC anti-Proliferative potential

AT-MSC, from healthy donors, were co-cultured with anti-CD3/CD28 stimulated allogeneic PBMC isolated from healthy or T2D subjects (Fig.3B). In the applied co-culture setting, AT-MSC moderately suppressed the proliferation of PBMC from healthy or T2D donor with a slightly weaker inhibitory effect in the diabetic milieu (Fig.3C).

3.4. Diabetic milieu significantly compromised AT-MSC modulatory effect on T Cell Activation Markers

Flow cytometer analysis of the activation markers, CD25 and HLA-DR on $CD3^+$ T cell demonstrated that presence of AT-MSC reduced the expression of the indicated markers. Gating strategy was lymphocyte gating based on FSC and SSC, $CD45^+$ leukocytes were then gated to further gate$CD3^+$ T cell, then double positive CD3 and CD25 or HLA-DR were defined. AT-MSC in co-culture with stimulated nPBMC or dPBMC reduced the expression of CD25 (Fig.4A) and HLA-DR (Fig.4B) on $CD3^+$ T cell. Importantly, AT-MSC in co-culture with dPBMC, relative to nPBMC, significantly had attenuated potential to modulate the

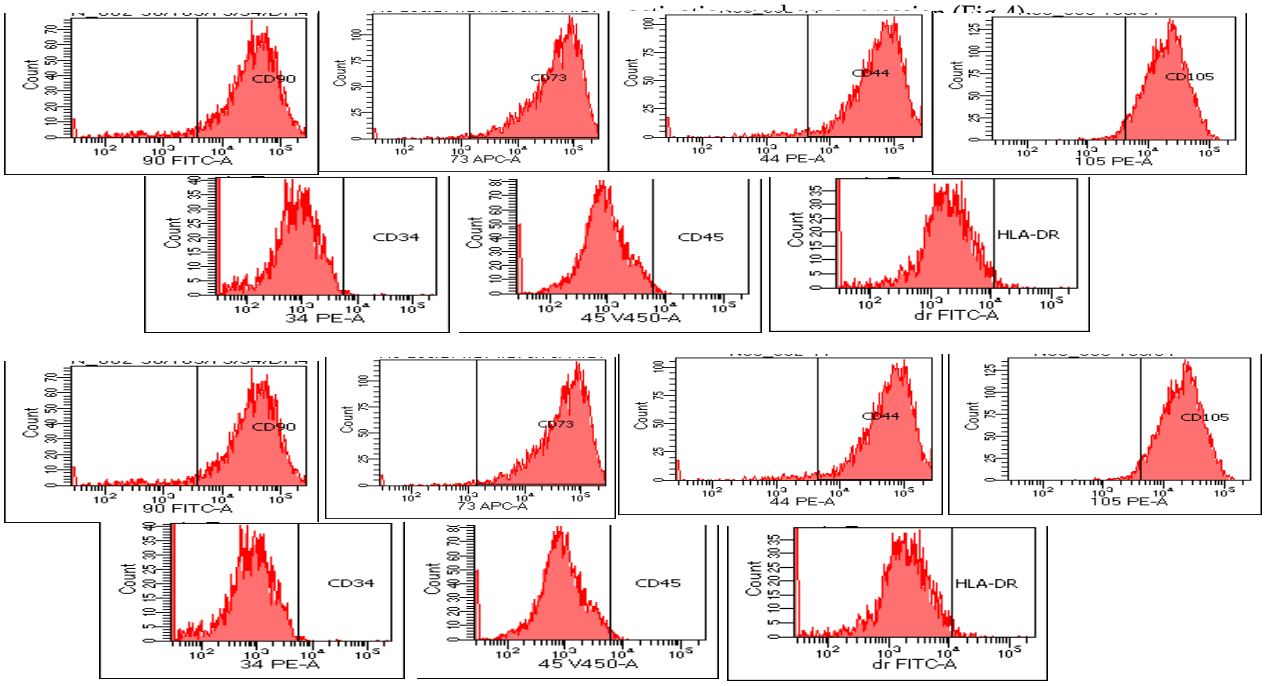

Figure 2. Immunophenotypic characteristics of nAT-MSC and dAT-MSC. (A) vs (B) represents flow cytometer histograms for CD44, CD73, CD90, CD105, CD45 and CD34 on nAT-MSC vs dAT-MSC, respectively.

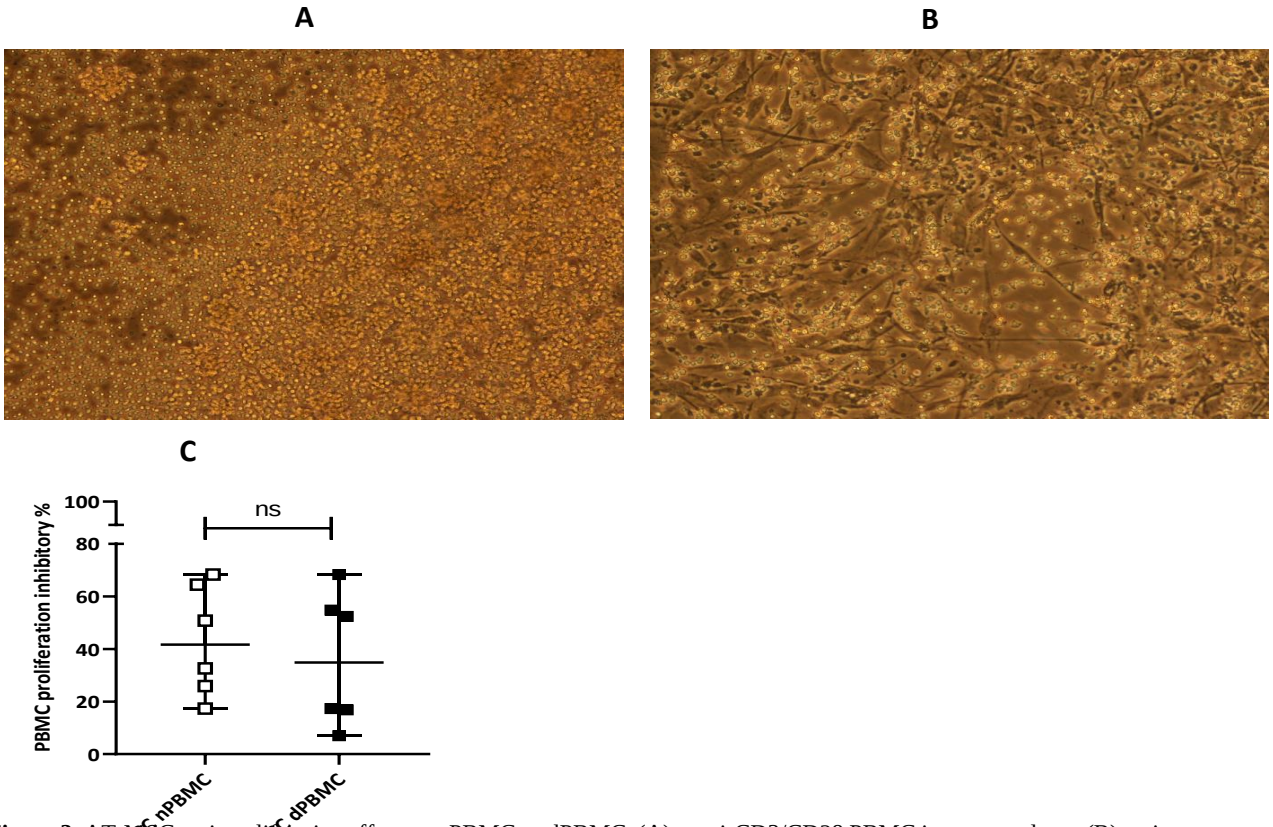

Figure 3. AT-MSC anti-proliferative effect on nPBMC vs dPBMC. (A) anti-CD3/CD28 PBMC in mono-culture. (B) anti-CD3/CD28 PBMC cultured on AT-MSC (PBMC/AT-MSC co-culture). Scale bar = 100 μm.(C) the percentage of stimulated nPBMC or dPBMC in the presence of nAT-MSC. For (C) Bar lines represent median. Mann-Whitney test was used for statistical analysis. CC abbreviates coculture

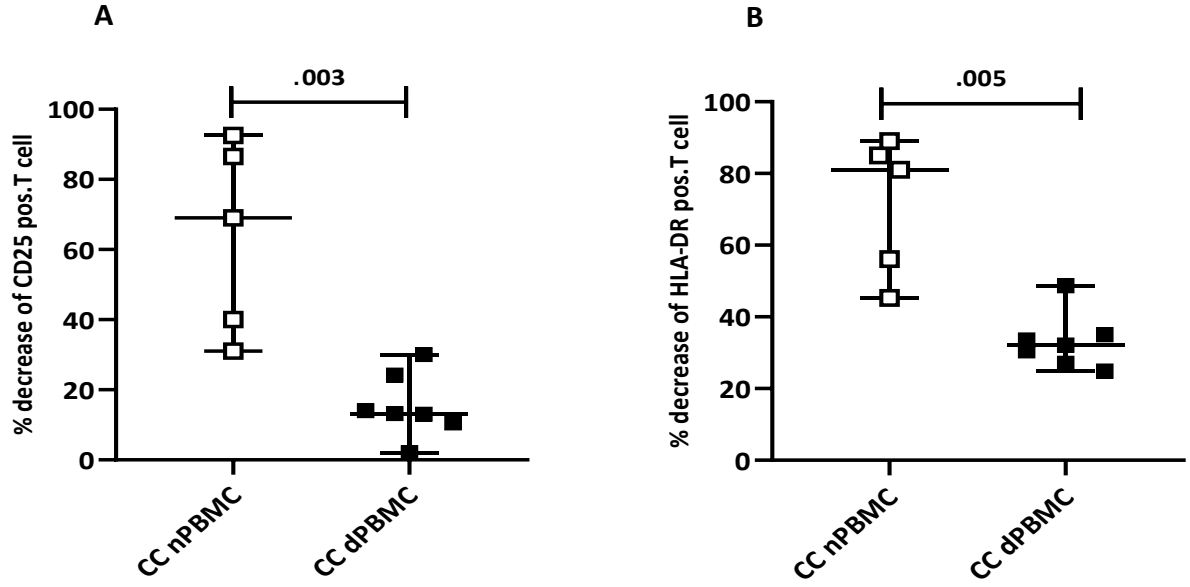

Figure 4. AT-MSC modulation of activation markers on nPBMC vs dPBMC**.** nAT-MSC, in co-culture, were able to suppress the expression of CD25 (A) and HLA-DR (B) on nPBMC vs dPBMC with significant reduced potential in the diabetic milieu. Bar lines represent medians. Mann-Whitney test was used for statistical analysis. CC abbreviates coculture

3.5. Diabetic milieu enhanced inflammation in the presence of AT-MSC

Preliminary results for expression analysis of the pro-inflammatory (*IFN-ɤ and TNF-a*) and the anti-inflammatory (*IL-4 and IL-10*) genes in anti-CD3/CD28 PBMC, from healthy or T2D donors, and co-cultured with nAT-MSC are illustrated (Table 3). Expression of the master pro-inflammatory cytokine genes, *IFN-ɤ*, and TNF-α, was upregulated in co-cultures with dPBMC by 1.5 > fold change <3. However, more samples are needed to confirm the results.

3.6. dAT - MSC had impaired anti-proliferative effect on autologous dPBMC

AT-MSC, from T2D donors (n=4), were co-cultured with anti-CD3/CD28 stimulated autologous PBMC. dAT- MSC did not significantly suppress, however, they enhanced the proliferation of autologous stimulated PBMC (Fig.5A). Importantly priming with IFN-ɤ improved the weak anti-proliferative effect in 3 out of 4 dAT-MSC lines (Fig.5B). Donor variation in response to IFN-ɤ to correct the anti-proliferative effect of AT-MSC on stimulated PBMC was observed. For IFN-primed dAT-MSC lines 1, 2, 3, 4, the immunosuppressive/anti-proliferative effect improved by 7%, 16%, 34% and 11%, relative to the respective non-primed dAT-MSC lines.

4. DISCUSSION

Hyperglycemia, oxidative stress and inflammation in T2D contribute to vascular and non-vascular complications causing socio-economic burdens[4, 29, 30]. Accordingly, approaches to dampen inflammation, immune dysregulation and oxidative stress are potentially recommended to improve glucose metabolism in T2D patients[2, 31]. AT-MSC are obtainable, expandable, multipotent cells with unique immunomodulatory and anti-oxidative properties, thus they are an ideal therapeutic modality in T2D[20].

Discordant results about the effect of diabetes mellitus, on the basic characteristics and functionality of MSC, from unchanged to negatively affected, have been reported and reviewed[32]. Recently, **Abu-Shahba *et al***. [23] has detected abnormalities of AT-MSC derived from T2D patients in the expression of surface and secretory immunomodulatory molecules. Among those abnormalities are attenuated expression of CD200, CD276 and IL-1 receptor antagonist (IL-1RA), while enhanced IL-1β, favoring a pro-inflammatory phenotype [23].

The present work aims to evaluate whether immune cells from T2D patients are sensitive to the inhibitory effects of allogeneic or autologous AT-MSC. Here we show that allogeneic AT-MSC can comparably inhibit the proliferation of anti-CD3/CD28- stimulated nPBMC and dPBMC, however, reduce the expression of CD25 and HLA-DR on dPBMC, significantly less potential than nPBMC. Additionally, autologous dAT-MSC, on the tested small sample scale, are not able to suppress the proliferation of dPBMC, however, priming of the cells with IFN-ɤ might promote the anti-proliferative effect of dAT-MSC.

The current study, microscopic follow up for cell morphology in culture showed that both nAT-MSC and dAT-MSC are adherent, proliferative cells with spindle fibroblastic morphology, as MSC have been characterized[6]. Immunophenotypic characterization of nAT-MSC and dAT-MSC reveals that both cell types exhibit the typical MSC surface CD profile[6], without significant differences between them. nAT-MSC and dAT-MSC are positive for CD90, CD105, CD44, and CD73 and negative for CD45, CD34 and HLA-DR. Such findings support earlier data[23-25, 33], while contradict others which report that AT-MSC isolated from T2D express higher levels of stemness/stromal marker CD90 than nAT-MSC[34].

The present study confirms the repeatedly reported anti-proliferative potential of AT-MSC from healthy donors on anti-CD3/CD28 stimulated PBMC [13-15]. The mechanisms administer AT-MSC anti-proliferative effect and immunosuppression vary to include direct cell-cell interactions *via* expression of adhesion and surface molecules as CD54[35]and CD200[36] and paracrine activity *via* secretion of soluble immunomodulators. Among the leading ones are indoleamine 2,3 dioxygenase 1 (IDO) [13-15]and prostaglandin E2 (PGE2) [27, 37, 38].Importantly, we reveal that the anti-proliferative effect of nAT-MSC on PBMC from T2D donors is slightly, but non-significantly, lower than their effect on PBMC from healthy subjects.

T lymphocytes by anti-CD3/CD28 activation upregulate the expression of a number of surface markers which are needed for T cell activation, proliferation and differentiation, among them are CD25 and HLA-DR[39, 40].In the present study, we demonstrate a downmodulation of the percentage of $CD3^+$ $CD25^+$ T cell in the presence of nAT-

MSC.CD25 is IL-2 receptor α -subunit and is closely linked to sensitivity of T cell to IL-2 which is needed to sustain T cell survival and proliferation[35,39].Mechanisms contribute to AT-MSC-mediated down-regulation of CD25 on lymphocytes including transcriptional inhibition and induction of receptor shedding have been introduced[41].

Table 3. Fold change of pro- and anti-inflammatory genes in nPBMC or dPBMC in co-culture with nAT-MSC

	IFN-ɤ	*TNF-a*	*IL-10*	*IL-4*
nPBMC CC	0.57	0.29	2.16	3.34
dPBMC CC	2.42	1.83	4	2.52

CC: abbreviates co-culture

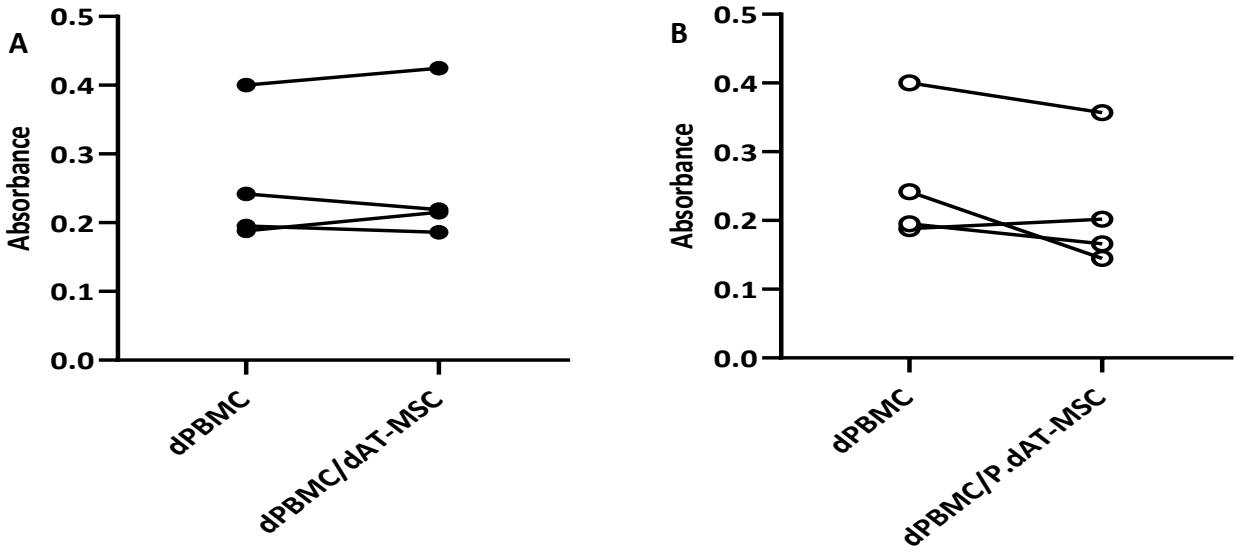

Figure 5. dAT-MSC anti-proliferative effect IFN-ɤ promoting effect on this potential. (A**)** dAT - MSC did not significantly suppress or even enhanced the proliferation, represented by absorbance, of autologous stimulated PBMC, in co-culture relative to dPBMC mono-culture. (B) IFN-ɤ priming of dAT-MSC. Bar lines represent medians. Mann-Whitney test was used for statistical analysis. P abbreviates primed with IFN-ɤ.

Allogeneic AT-MSC, in our study, down-regulated the percentage of $CD3^+$ $CD25^+$ T cell fraction of nPBMC *vs* dPBMC by a median percentage of 69% (n=5) *vs* 7.4% (n=7), respectively. Such finding suggests that the milieu of T2D strongly compromises the potential of AT-MSC to down-modulate CD25 expression and accordingly activation of PBMC. We also monitored the expression of the late activation marker HLA-DR, which causes the involvement of immune cells in the "graft-versus-host" reaction[42]. Allogeneic nAT-MSC reduce its expression with a significantly attenuated potential in co-culture with dPBMC, relative to nPBMC. Previous studies have demonstrated the inhibitory effect of AT-MSC/PBMC on HLA-DR expression by activated $CD3^+$ T cell[42, 43].

In addition to the defective AT-MSC potential to modulate T cell activation markers in T2D environment, we have detected that AT-MSC enhance the gene expression of pro-inflammatory cytokines *TNF-a* and *IFN-ɤ* in dPBMC, not in nPBMC. A previous study has reported that pre-stimulation of T lymphocytes 48h before the co-culture with AT-MSC impairs the capacity of AT-MSC to inhibit T cell proliferation. The authors have proposed that when AT-MSC would encounter and interact with already activated immune cells, derived from patients with an inflammatory disease, their immunomodulatory capacity might be affected[44]. Such proposal might explain the attenuated immunosuppressive effect, in our study, of allogeneic healthy AT-MSC on PBMC derived from patients with T2D.Data reported here augment the

need to evaluate the inflammatory status of the patient before recruitment and implementation in AT-MSC treatment to ensure favorable clinical outcome.

Our study also demonstrates that AT-MSC isolated from T2D patients are not able to suppress the proliferation of stimulated autologous PBMC. Few studies have demonstrated that the anti-proliferative effect of AT-MSC, derived from T2D [25]or T2D and obese [24] patients on allogeneic-stimulated PBMC, is detrimentally impacted. Lower basal expression of the anti-inflammatory cytokine TGF-β by dAT-MSC than nAT-MSC has been attributed as the mechanism underlying the defect in dAT-MSC immunosuppressive functions[24].

Importantly, our preliminary results have showed that pre-conditioning with IFN-ɤ might enhance the anti-proliferative effect on autologous PBMC. Numerous studies provide evidence that priming of AT-MSC with a pro-inflammatory stimuli as IFN-ɤ enhance the immunosuppressive[12-15], no the immunogenic[45], functions of AT-MSC. In a previous study, we have demonstrated that nAT-MSC and dAT –MSC respond similarly to IFN-ɤ, upregulating important immunomodulators as the tryptophan catabolizing enzyme IDO, the anti-inflammatory cytokine IL-1RA, while, down regulating the pro-inflammatory cytokines IL-1β and TNF-α[23].The indicated molecules mediate the immunosuppressive actions of AT-MSC on stimulated T cell proliferation, activation and functions[11, 20].

CONCLUSION

The results suggest that T2D might negatively affect the immunosuppressive potential of AT-MSC and pre-conditioning of dAT-MSC with a pro-inflammatory stimulus like IFN-ɤ could enhance their therapeutic effect. IFN-ɤ mediated expression of IDO, among other immunomodulators, may implicate the enhanced AT-MSC immunosuppression in inflammation**.**

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Conflicts of Interest: The authors declare no conflicts of interest

Ethical Statement: This study was approved by the Local Medical Research Ethics Committee of the National Research Centre, Cairo, Egypt (Registration no. 16079).

Author Contribution: M.M.: contributed to conception and experimental design, performing experiments, data collection and interpretation, manuscript writing; N.A: contributed to experimental design, performing experiments and interpreting results. G.N: contributed to interpreting molecular results. K.A: contributed to revising the molecular work and reviewed the manuscript. R.G: contributed to flow cytometer experiments. A.E: contributed to obtaining blood and adipose tissue samples. O.A: contributed to conceptualization of the study, supervising the experiments, and financial support. A.A: supervised plan of the work and reviewed the manuscript.

Limitations and Perspectives: Larger healthy and T2D sample sizes are needed to confirm the results. Further experiments to elucidate the effect of AT-MSC on the differentiation of T lymphocytes in T2D *via* studying the gene and protein levels of the master cytokines of T cell subsets may be considered in the near future.

List of Abbreviations

T2D	Type 2 Diabetes
AT-MSC	Adipose Tissue-derived Mesenchymal stem/stromal cell
PBMC	Peripheral Blood Mononuclear Cells
Brdu	5-bromo-20-deoxyuridine
IR	Insulin Resistance
ISCT	International Society of Cellular Therapy
IFATS	International Federation for Adipose Therapeutics and Science
CD	Cluster Differentiation
PBS	Phosphate-Buffer Saline
FBS	Fetal Bovine Serum
BM	Basal Medium
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
18S	18S ribosomal RNA
IL-1RA	IL-1 receptor antagonist
IDO	Indoleamine 2, 3 Dioxygenase 1
PGE2	Prostaglandin E2
MLR	Mixed Lymphocyte Reaction
vs	Versus
P	Passage

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