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Th17 immune response to adipose tissue-derived mesenchymal stromal cells

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Abstract

Adipose tissue-derived mesenchymal stromal cells (ASCs) hold the promise of achieving successful immunotherapeutic results due to their ability to regulate different T-cell fate. ASCs also show significant adaptability to environmental stresses by modulating their immunologic profile. Cell-based therapy for inflammatory diseases requires a detailed understanding of the molecular relation between ASCs and Th17 lymphocytes taking into account the influence of inflammation and cell ratio on such interaction. Accordingly, a dose-dependent increase in Th17 generation was only observed in high MSC:T-cell ratio with no significant impact of inflammatory priming. IL-23 receptor (IL-23R) expression by T cells was not modulated by ASCs when compared to levels in activated T cells, while ROR-γt expression was significantly increased reaching a maximum in high (1:5) unprimed ASC:T-cell ratio. Finally, multiplex immunoassay showed substantial changes in the secretory profile of 15 cytokines involved in the Th17 immune response (IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-22, IL-21, IL-23, IL-25, IL-31, IL-33, IFN-γ, sCD40, and TNF-α), which was modulated by both cell ratio and inflammatory priming. These findings suggest that Th17 lymphocyte pathway is significantly modulated by ASCs that may lead to immunological changes. Therefore, future ASC-based immunotherapy should take into account the complex and detailed molecular interactions that depend on several factors including inflammatory priming and cell ratio.

KEYWORDS
adipose stem cells, cytokines, IL-23, Th17

1 | INTRODUCTION

Adipose tissue-derived mesenchymal stromal cells (ASCs) are considered a major breakthrough in the field of cell-based therapy due to their remarkable therapeutic and regenerative properties

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(Frese, Dijkman, & Hoerstrup, 2016; Gimble et al., 2013). Compared with other sources, ASCs show significant advantages including an easy-to-reach tissue, important potential cell yield, and less morbid isolation and harvesting procedures from patients (Feisst, Meidinger, & Locke, 2015). The International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) have established the minimal definitions for in vitro culture and expansion of adipose tissue-derived mesenchymal stem cells (Bourin et al., 2013). ASCs showed some biological differences according to the number of passages or the anatomic location from which fat is removed (Di Battista et al., 2014; Di Taranto et al., 2015). They are immunoprivileged by lacking HLA-DR and costimulatory signaling pathways, and in parallel display strong immunomodulatory properties (Fayyad-Kazan, Faour, Badran, Lagneaux, & Najar, 2016; Puissant et al., 2005). To control immune cell responses, mesenchymal stem cells (MSCs) either produce a multitude of immunoregulatory factors or modulate the immunological cytokine pathways of targeted immune cells (Glenn & Whartenby, 2014). Thus, MSCs can modulate targeted immune cells either directly or indirectly by influencing the activities of other immune cells (Kyurkchiev et al., 2014). Of interest, T lymphocytes represent an important cellular target for cell-based therapy. Interleukin 17 (IL-17) producing T cells are mainly involved in the etiology of autoimmune and inflammatory disorders (Angelotti et al., 2017; Maddur, Miossec, Kaveri, & Bayry, 2012; Middleton, Annels, & Pandha, 2012). Accordingly, Usha Shalini et al. showed that ASCs can alter cytokines production from T cells, B cells, and Treg during an anti-inflammatory immune response (Usha Shalini, Vidyasagar, Kona, Ponnana, & Chelluri, 2017). The differentiation of naïve $CD4^+$ T cells toward Th17 cells is regulated by multiple cytokines released during the inflammatory process (Iwakura, Ishigame, Saijo, & Nakae, 2011). Interleukin-23 receptor (IL-23R) and the retinoic acid receptor-related orphan nuclear receptor (ROR-γt) play a crucial role in the development and the maintenance of Th17 cells (Ivanov et al., 2006). Therefore, studying the immune response of Th17 cells in the presence of ASCs, as well as the influence of inflammatory priming and cell ratio on Th17 immune response modulation is important to achieve successful cell-based therapy. In particular, we analyzed the modulation of IL-17A, IL-23R, and ROR-γt expression as well as the Th17 cytokine pathway profile (multiplex immunoassay) in cocultures of ASCs and T cells. Collectively, our results demonstrate that cell ration and inflammation significantly modulate Th17 cell immune response by ASCs. The expression and the secretion of the main Th17-associated factors are thus fine-tuned and should be well-understood and analyzed to ensure safe and efficient ASCs-based therapy particularly in the presence of immunological or inflammatory contexts.

2 | MATERIALS AND METHODS

2.1 | Isolation and culture of ASCs

Freshly obtained lipoaspirates were processed to isolate ASCs as previously described (Busser et al., 2014). Briefly, the lipoaspirates were digested in an equal volume of collagenase D (0.1%; Roche, France) for 30 min at 37°C. After digestion, an equal volume of phosphate-buffered saline (PBS)–EDTA was added to inhibit collagenase D enzymatic activity and to wash the sample by centrifugation for 5 min at 80 g speed. The upper layer containing the aqueous phase and adipocytes were discarded. The pellet composed of the stroma-vascular fraction was then plated in culture flasks containing Dulbecco's modified Eagle's medium (DMEM) with 1.0 g/L glucose, without L-glutamine (Lonza, Bazel, Switzerland) supplemented with 15% fetal bovine serum (Sigma, MO), 2 mM L-glutamine (Lonza), and 1% pen/strep amphothericin B solution (Lonza). After 5 days of culture in a humidified atmosphere, 5% CO_2 at 37°C, the culture dishes were washed with culture media and media changing was done on a weekly basis. Cells were kept in culture until 80–90% of confluency. When subconfluency was reached, adherent cells were harvested by TrypLE Select (Gibco, MA; 10 min, 37°C), washed in sterile warm PBS 1 ×, counted and analyzed.

2.2 | Inflammatory priming of ASCs

Inflammatory priming of ASCs was achieved as previously described (Raicevic et al., 2010). The cells were incubated overnight with a cocktail of proinflammatory cytokines that includes 25 ng/ml IL-1β, 103 U/ml IFN-γ, 50 ng/ml TNF-α and 3×10^3 U/m IFN-α (Peprotech, Rocky Hill, NJ)

2.3 | ISCT compliance of ASCs

ASCs were defined as proposed by the ISCT committee. Briefly, their phenotype was determined by flow cytometry (MACSQuant, Miltenyi Biotec, Germany) using the following monoclonal antibodies: anti-CD45-FITC and anti-HLA-DR-PE (Exalpha Biologicals, Maynard, MA), anti-CD73-PE were purchased from (BD Biosciences, San Diego, CA), anti CD14-PE, anti-CD19-PE, anti-CD105-FITC, and anti-CD90-PE were purchased from (R&D systems, Minneapolis, MN). The tri-lineage capacity of ASCs was confirmed by inducing their differentiation into adipogenic, osteogenic and chondrogenic lineages using appropriate culture conditions (NH media; Miltenyi Biotec).

2.4 | T cells

The peripheral blood of healthy donors who gave informed consent have undergone a Ficoll-Hypaque gradient centrifugation to obtain peripheral blood mononuclear cells (PBMCs). T cells were purified (> 95% purity) by positive selection using the MACS system (Miltenyi Biotec) and then activated by using a cocktail of phytohemagglutinin (PHA, 5 μg/ml; Remel, Belgium) and IL-2 (20 U/ml; Biotest AG, Germany). Activated T cells and inflammatory primed or not ASCs were incubated during 5 days in cocultures at both 1:80 and 1:5 cell ratio.

2.5 | Identification of Th17 cells

Th17 cells were identified by flow cytometry by determining the percentage of IL-17 secreting T cells (BD Pharmingen™, NJ) and both

the expression of ROR-γt (R&D Biosystems, Minneapolis, MN) and IL-23 receptor (R&D Biosystems) according to the manufacturer instructions.

2.6 | Th17 cytokine pathway

The network of cytokines involved in the immunobiology of Th17 cells was analyzed by using the Bio-Plex Pro™ Human Th17 Cytokine Assays® (Bio-Rad Laboratories, Inc., CA) according to the manufacturer's instructions. This assay enables robust and reproducible measurement of the following cytokines (IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFNγ, soluble CD40 ligand (sCD40L), and TNF-α) from different culture supernatants.

2.7 | Flow cytometry

The data were acquired and analyzed on a MacsQuant analyzer (Miltenyi Biotec).

2.8 | Statistics

Data are expressed as mean ± standard error of the mean. Statistical analysis was performed using Prism software for paired samples. Statistical significance was accepted as determined by ANOVA for repeated measures followed by a Newman–Keuls multiple comparison test. A value of $*p < 0.05$ was considered statistically significant, $**p < 0.01$ or $***p < 0.001$.

2.9 | Ethical approval

All procedures were performed in the study and the involved human participants were in accordance with the ethical standards of the Local Ethics Committee of the "Institut Jules Bordet" (Belgium) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Lipoaspirates were obtained from healthy patients undergoing esthetic surgical procedures after giving informed written consent.

3 | RESULTS

3.1 | ASCs comply with ISCT rules

ASCs were defined according to the guidelines established by the ISCT. In cultures, ASCs were likely fibroblastic and highly adherent to plastic. They demonstrated capacities to differentiate into osteoblastic, chondrogenic and adipogenic lineages. Finally, flow cytometry analysis revealed the expression of the mesenchymal stem cell markers CD73, CD90, and CD105 and the absence of the hematopoietic stem cell markers CD45, CD14, CD19, CD34, and HLA-DR (data not shown). Then, we assessed the effects of ASCs cultured in two cell ratios (low vs. high) and in the presence or not of inflammation on Th17 immunobiology.

3.2 | ASCs modulated IL-17A expression

IL-17A expression analyzed by flow cytometry was used as an indicator of Th17 cell generation. The proportion of Th17 in unactivated T cells (0.3450 ± 0.09566) increased after T-cell activation with PHA/IL-2 (0.5617 ± 0.1246). Cell ratio of T cells to MSCs in the cocultures but not inflammation had a great impact on Th17 proportion as a 1:5 ratio showed significant and substantial increase in Th17 proportion (4.272 ± 0.8896) when compared with 1:80 ratio (1.613 ± 0.06346). Inflammation only caused a moderate and significant increase in Th17 proportion in cocultures at 1:5 ratio (4.880 ± 0.7351) when compared with those not exposed to inflammation (4.272 ± 0.8896) (Figure 1).

3.3 | ASCs induce ROR-γt expression

PHA/IL2-activated T cells (15.59 ± 2.4943857) showed significantly lower levels of ROR-γt than their unactivated counterpart (25.8333333 ± 1.74913312). In the presence of activated T cells (15.59 ± 2.4943857), ASCs at both 1:80 and 1:5 ratio either primed (21.9683333 ± (3.64468609; 20.6116667 ± 1.26809174) or not (20.463333 ± 1.10355184; 24.76 ± 1.0907612) with inflammation demonstrated significantly higher ROR-γt expression levels (Figure 1).

3.4 | ASCs do not modulate IL-23 receptor expression

Flow cytometry analysis revealed that PHA/IL-2-activated T cells 15.625 ± 1.369478 showed significantly elevated IL-23 receptor (IL-23R) expression levels when compared to levels in unactivated T cells (2.08833333 ± 0.69542553). Interestingly, IL-23R expression levels in PHA/IL-2-activated T cells were not modulated by either constitutive or inflammatory primed ASCs irrespective of the used cell ratio (1:5 and 1:80) in cocultures (Figure 1).

3.5 | ASCs altered Th17 cytokine pathway

We finally investigated the profile of the main cytokines involved in Th17 signaling pathway in ASCs (cell ratio and inflammatory priming) and PHA/IL2-activated T cells cocultures (Figure 2).

3.5.1 | T cells and activation

PHA/IL-2-activated T cells secreted almost all the cytokines involved in Th17 signaling pathway except IL-25 (1.9425 ± 0.21) and IL-33 (11.00 ± 2.45) which were found at very low levels. In comparison to unactivated T cells, PHA/IL-2-activated T cells, respectively, secreted substantial levels of IL-1β (17.17 ± 1.05; 0.37 ± 0.07), IL-4 (597.28 ± 57.44; 0), IL-6 (598.785 ± 48.86;7.98 ± 0.40), IL-10 (402.83 ± 54.01; 0), IL-17A (305.91 ± 33.17; 4.5575 ± 0.85), IL-17F (365.42 ± 36.45;9.43 ± 2.37), IL-21 (266.41 ± 18.53), IL-22

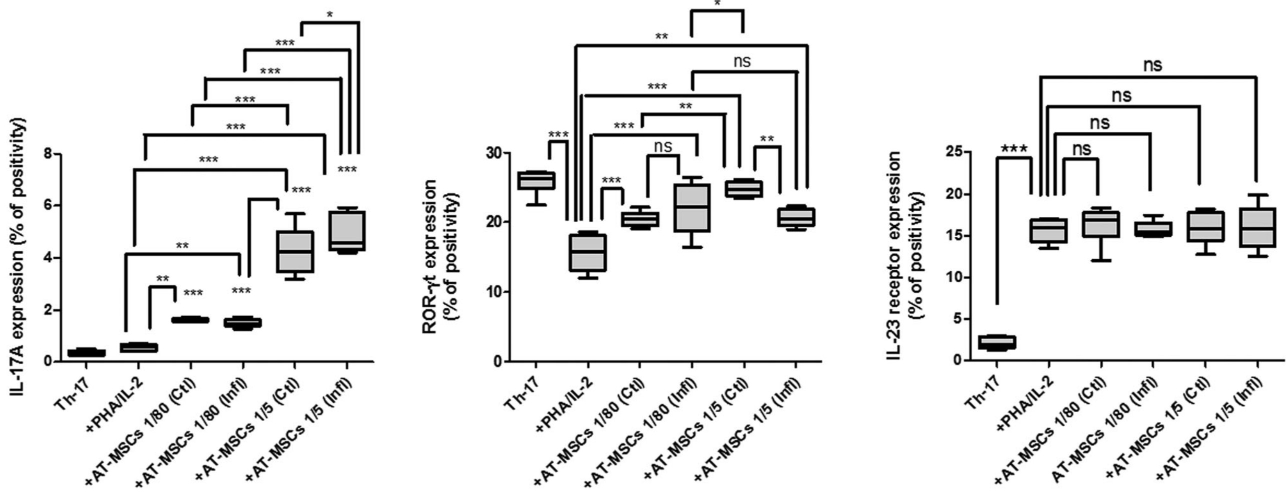

FIGURE 1 Influence of ASCs on IL-17A, IL-23 receptor, and ROR-γt expression. ASCs are cocultured at (1:5) or (1:80) cell ratio with either unactivated or PHA/IL-2 activated T cells as indicated. Levels of IL-17A, ROR-γt, and IL-23 receptor expression was analyzed by flow cytometry. Data are expressed as percentage of control ± *SEM* from four to five independent experiments. **p* < 0.05, ***p* < 0.01, ****p* < 0.01 versus control. ASC: adipose tissue-derived mesenchymal stromal cell; IL: interleukin; PHA: phytohemagglutinin; ROR-γt: retinoic acid receptor-related orphan nuclear receptor

(157.11 ± 12.19; 8.10 ± 1.08), IL-23 (103.89 ± 3.77; 53.53 ± 5.34), IL-31 (711.21 ± 8.12; 5.69 ± 0.720), IFN-γ (3508.20 ± 284.83; 11.54 ± 0.59), sCD40 (896.49 ± 8.29; 19.85 ± 3.33) and TNF-α (1587.91 ± 92.17).

3.5.2 | ASCs and inflammatory priming

In contrast to PHA/IL-2-activated T cells, ASCs do not constitutively release a broad range of Th17 related cytokines. Irrespective of the cell ratio (1:80 or 1:5), nonprimed ASCs displayed insignificant levels

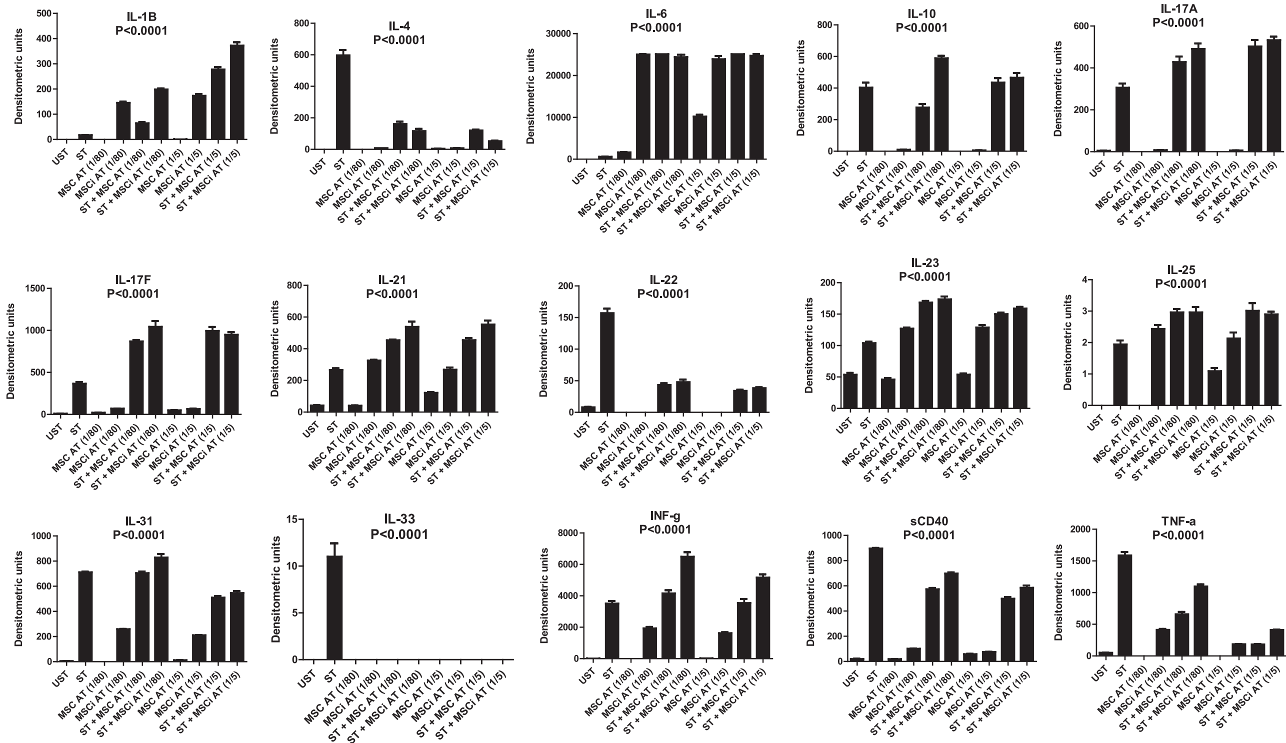

FIGURE 2 Profile of Th17-associated cytokines produced in high (1:5) and low (1:80) ASC:T-cell ratio cocultures. ASCs are cocultured at (1:5) or (1:80) cell ratio with either unactivated or PHA/IL-2 activated T cells as indicated. Levels of the different cytokines were analyzed as indicated in the materials and methods section. Data are expressed as percentage of control ± *SEM* from four to five independent experiments. **p* < 0.05, ***p* < 0.01, ****p* < 0.01 versus the corresponding control. ASC: adipose tissue-derived mesenchymal stromal cell; IL: interleukin; PHA: phytohemagglutinin

of IL-1β, IL-4, IL-10, IL-17A, IL-22, IL-25, IL-31, IL-33, IFN-γ, and TNF-α. Unlike low cell ratio, high cell ratio of ASCs allowed considerable levels of secreted IL-6 (1648.49 ± 154.46; 10179.40 ± 856.79), IL-17F (20.30 ± 3.05; 49.45 ± 5.15), IL-21 (40.32 ± 5.19; 121.51 ± 9.62), IL-23 (45.95 ± 4.88; 53.76 ± 4.08), and sCD40 (18.38 ± 1.52; 58.31 ± 5.08), respectively.

Independently of the cell ratio, inflammatory priming either induced or increased the secretion of distinct Th17 related cytokines. The levels of IL-4, IL-10, IL-17A, IL-22, IL-25, and IL-33 were irrelevant and similar to those of nonprimed MSCs. In contrast, inflammatory primed ASCs at both low (1:80) and high (1:5) cell ratio nearly showed the same increase in the levels of IL-1β (145.03 ± 7.88; 173.22 ± 11.41), IL-6 (25007.45 ± 127.99; 23898.80 ± 1284.55), IL-17F (68.81 ± 1.9; 64.36 ± 7.10), IL-21 (325.86 ± 7.85; 267.57 ± 24.12), IL-23 (126.84 ± 3.27; 128.69 ± 6.74), IL-31 (258.97 ± 4.12; 210.81 ± 3.11), IFN-γ (1930.91 ± 159.88; 1800.00 ± 106.21), sCD40 (100.84 ± 4.42; 75.65 ± 3.18), and TNF-α (412.99 ± 24.20; 185.57 ± 4.97).

3.5.3 | Cocultures

Cocultures of ASCs and PHA/IL-2-activated T cells distinctively changed the profile of the cytokines related to Th17 signaling pathway. Furthermore, inflammatory priming of ASCs as well as the cell ratio strongly influenced the levels of each cytokine.

Low cell ratio coculture: When comparing nonprimed versus inflammatory primed ASCs at (1:80) cell ratio in cocultures with PHA/IL-2-activated T cells, we observed significantly increased levels of IL-1β (64.44 ± 8.05; 198.39 ± 7.82), IL-6 (25081.34 ± 0; 24393.90 ± 961.19), IL-17A (428.16 ± 52.16; 490.93 ± 45.61), IL-17F (868.98 ± 34.85; 1042.93 ± 120.18), IL-21 (453.61 ± 7.78; 538.23 ± 57.30), IL-23 (168.38 ± 5.49; 173.64 ± 7.59) and IFN-γ (4159.09 ± 396.44; 6487.21 ± 50472). The levels of IL-4 (161.02 ± 28.72; 116.31 ± 23.90), sCD40 (573.15 ± 21.43; 698.05 + 15.44), and TNF-α (659.74 ± 73.82; 1097.62 ± 57.32) were however significantly lower than those in PHA/IL-2-activated T cells. Cocultures at (1:80) cell ratio of PHA/IL-2-activated T cells with nonprimed ASCs showed lower levels of IL-10 (276.92 ± 45.05) and IL-31 (704.32 ± 27.51) while with inflammatory primed ASCs the levels of IL-10 and IL-31 were enhanced, respectively (588.81 ± 27.14 and 828.39 ± 49.26), in comparison to levels in PHA/IL-2-activated T cells alone (402.83 ± 54.01; 711.21 ± 8.12).

High cell ratio coculture: When comparing nonprimed versus inflammatory primed ASCs at (1:5) cell ratio in cocultures with PHA/IL-2-activated T cells, the levels of IL-1β (277.88 ± 17.68; 372.41 ± 22.93), IL-6 (25081.34; 24695.71 ± 667.93), IL-10 (435.33 ± 55.35; 465.77 ± 51.85), IL-17A (502.68 ± 60.77; 532.61 ± 28.92), IL-17F (994.11 ± 94.95; 948.11 ± 53.36), IL-21 (454.33 ± 25.52; 552.95 ± 43.20) and IL-23 (150.01 ± 4.45; 158.89 ± 4.61) were consistently increased. When compared to levels in PHA/IL-2-activated T cells alone, reduced levels of IL-4 (120.62 ± 8.72; 52.23 ± 5.21), IL-22 (34.20 ± 2.82; 38.49 ± 2.19), IL-31 (509.64 ± 23.94; 545.84 ± 26.82), sCD40 (497.83 ± 26.30; 584.37 ± 31.83), and TNF-α (183.27 ± 13.42; 411.30 ± 7.56) were observed. IFN-γ levels were significantly induced in cocultures of PHA/IL-2-activated T cells with inflammatory primed ASCs (5162.42 ± 363.24) and were higher to the levels found in the nonprimed counterpart (3541.89 ± 519.00). The levels of IL-25 (3.01 ± 0.49; 2.90 ± 0.15) and IL-33 (3.11 ± 6.22; 0) remained negligible in such conditions.

4 | DISCUSSION

The cellular and molecular environment are critical during lymphocytes differentiation into distinct T-cell subsets including T-helper 1 (Th-1), Th-2, Th-9, Th17, or regulatory T cells (Treg) and therefore important for the immunological response. The immunomodulatory effect of MSCs even though well-established is still incompletely understood and need more critical analysis. While MSCs isolated from different types of tissues (e.g, bone marrow, cord blood, fat tissues, and so forth) showed similar-to-compatible properties, they greatly differ in their ability to immunomodulate T cells. Th17 cells which are actively involved in the process of tissue injury and inflammation, can be targeted by MSCs as a therapeutic strategy. Most recent evidence showed that MSCs can exert multiple immunomodulatory effects on the same type of immune cells according to the local environment or disease state, the MSCs injected dose (ranging from 0.5×10^6 to 10×10^6/kg of the recipients and higher), and the frequency of injection (single vs. multiple injections; Gao et al., 2016). Accordingly, Najar et al. (2009) have previously shown that the cell ratio used during in vitro coculture has a relevant impact on the immune response of MSCs (Najar et al., 2009).

Therefore, uncovering the immune response of Th17 cells to ASCs is relevant to achieve successful cell-based therapy. Accordingly, we observed that ASCs are capable of modulating the immunobiology of Th17 cells by predominantly affecting their signaling pathway.

In the current study, the cytokine profile released in the ambient media of ASCs and T cells can be theoretically subdivided into three groups. The first group of cytokines is crucial in inducing the differentiation of naive $CD4^+$ into Th17 which includes IL-1β and IL-23 and possibly TGF-β. The second group includes factors such as IL-6, IL-1, and TNF and are involved in maintaining T cells differentiation into inflammatory Th17 subsets. The last group of factors which is mostly restricted to IL-10, inhibits to limit additional differentiation and expansion of Th17. IL-23 which is necessary for commitment into Th17 phenotype can enhance Il-17A production and inhibit the release of both IL-10 and IFN-γ (Korn, Bettelli, Oukka, & Kuchroo, 2009; Miossec, Korn, & Kuchroo, 2009; Pandolfi, Cianci, Pagliari, Landolfi, & Cammarota, 2009). Interestingly, the immunomodulatory properties of MSCs are not spontaneously acquired but need to be primed with a set of proinflammatory factors, for example, IFN-γ, TNF-α, IL-1α, IL-1β, or IL-17 (Krampera et al., 2006; Sheng et al., 2008). However, sustained exposure will possibly, through the

activity of specific danger signal (Waterman, Tomchuck, Henkle, & Betancourt, 2010), irreversibly contributes to the development of inflammatory niche that further enhances differentiation and proliferation of Th17 subsets. In keeping with the inflammatory phenotype of differentiated T cells, we found that Th17 markers including ROR-γt expression and IL-17A release were greatly induced in coculture of ASC:T-cell ratio which also coincides with a significant upregulation in IL-6 production and a decrease of TNF release in the same types of cocultures. Accordingly, Chen et al found that pre-activation of MSCs with a set of regenerative and immunomodulatory factors typically TNF-α maximizes the therapeutic effect of MSCs in a model of intestinal injury (Chen et al., 2015). Therefore, a reduction in TNF-α in the cocultures and an upregulation in IL-6 production would further enhance an inflammatory niche favoring Th17 differentiation. Efficient increase of IL-17A expression by ASCs may correspond to a potential expansion of Th17 in the cocultures. Importantly, the levels of IL-23R remained unchanged but the levels of the anti-inflammatory IL-10 slightly increased and remained elevated. IL-17, ROR-γt, and IL-22 signaling through the IL-22R are known as key markers in mediating naïve $CD4^+$ differentiation and maintenance of Th17, while the release of a significant amount of IL-10 will oppose to such mechanism (Zhang, Yuan, Cheng, & Guo, 2011). In our study, the levels of IL-1 and IL-6 were significantly induced by primed ASCs oppositely to levels of TNF which were found to be very low. Furthermore, levels of IL-10 were also increased by primed MSCs while significant amount of IL-33 was still detected in the cocultures. These results confirm that the immunomodulatory activity of MSCs necessitates an inflammatory process transiently achieved by the generation of Th17 which will be further controlled by the release of large amount of anti-inflammatory cytokines mainly IL-10. Accordingly, Pricola, Kuhn, Haleem-Smith, Song, and Tuan (2009) showed that a priming signal induced by IL-6 is needed to maintain bone marrow–derived mesenchymal stem cells stemness and confers protection from apoptosis as well as being necessary to increase MSCs proliferation (Pricola, Kuhn, Haleem-Smith, Song, & Tuan, 2009). However, an imbalance in the cytokine profile toward sustained differentiation of Th17 and continuous release of proinflammatory cytokines can overcome the inhibitory effect of IL-10 and therefore create an inflammatory rather than an immunotolerant environment (Korn et al., 2009). Also, these cytokines are important in promoting MSCs migration to damaged tissues.

These results suggest that MSCs contribute to maintain and stabilize an already engaged Th17 cell population. Therefore, variations in such surrounding can induce MSCs to stimulate the differentiation of naïve CD4 + into Th17 subsets known to play key role in inflammatory and chronic diseases (Mills, 2008). As previously mentioned, the immunomodulatory properties of engrafted MSCs are tightly related to their proportion in the host organ. Variations in MSCs proportion can have profound opposing effects of either inhibiting or inducing inflammatory signals when exposed to immune cells. The latter response can be dictated by either inducing or inhibiting the differentiation of naïve T cells toward inflammatory Th17 cells. The retinoic acid receptor-related orphan nuclear receptor (ROR-γt), which is a master Th17 regulator, and IL-23R play an important role in Th17 differentiation and maintenance and profoundly modulate the expression of Th17-associated markers including IL-17A, IL-17F, and IL-23 (Muranski & Restifo, 2013). In conclusion, induction of ROR-γt and IL-23R expression will induce naïve T cells to exhibit an inflammatory phenotype characterized by the production of IL-17, IL-17F, IL-6, and TNF-α, but not IFN-γ and IL-4. Thus, modulation of ROR-γt expression by MSCs is a key factor in affecting the differentiation of T cells into pathogenic Th17 cells. Our data showed that, ASCs whether at low or high ratio, primed or not, are able to induce T cells in cocultures to express ROR-γt but not IL-23R. The latter results demonstrated that MSCs mediating induction of ROR-γt expression by activated T cells can shift the balance toward the generation of specific Th17 population. We further assessed the expression levels of IL-23R in cocultures at both (1:5) and (1:80) cell ratio with and without inflammatory conditions. IL-23R is activated by IL-23 which is an important cytokine involved in the differentiation of naive $CD4^+$ T cells into a pathogenic helper T cells (Th17/ThIL-17) subset.

In summary, ASCs may mostly modulate the expansion of Th17 through IL-6 and IL-1, in place of IL-23 (Guo et al., 2009). The inability of ASCs to modulate IL-23 receptor expression could be explained by the low levels of IL-4 and high IFN-γ levels in these conditions. IL-4 and IFN-γ oppositely to IL-6 are known to downregulate the expression of IL-23R. While the expression of IL-17, ROR-γt and IL23R is important for Th17 differentiation, maintenance, and expansion of the differentiated Th17 subsets depend upon the cytokine profile released in the T cells surrounding. To this end, we evaluated the cytokine profile known to participate in modulating T cells differentiation and activity in cocultures with either primed or not primed ASCs. Interestingly, we found that the cytokine profile known to play a crucial role in generating Th17 including IL-6, IL-21, IL-1β, and IL-23 are modulated in cocultures of ASCs and T cells and in a dose-dependent manner. We believe that transient production of these cytokines although contributing to the generation of Th17 cells, will first enhance the immunomodulatory properties of ASCs characterized by the production of sustained anti-inflammatory cytokines, for example, IL-10.

Finally, the plasticity of MSCs in modulating the immune response is a key element toward establishing successful cell transplant therapy. Additionally, the differentiation of T cells into pathogenic Th17 is an important parameter that requires considerable attention when planning stem cell engraftment since biologic alterations of MSCs function might contribute to sustained differentiation of pathogenic Th17 rather inducing an immunosuppressive response.

This plasticity which may participate in the protection against microbes, is involved in developing autoimmunity, and is important for the antitumor activity of Th17 cells in models of adoptive cell transfer therapy (Muranski & Restifo, 2013). In conclusion, our finding showed that ASCs promote the development of a proinflammatory Th17 phenotype from activated T cells which is opposite

to the well-documented immunosuppressive effect of MSCs. These discrepancies evolve from incomplete knowledge about the mechanisms modulating MSC-Th17 lymphocyte interactions, which have crucial implications in the use of MSCs in clinical setting. Given the role of Th17 cells in human diseases (Wang et al., 2015), the activation and the differentiation state of T cells as well as the cell ratio, expansion passage and priming MSCs with inflammation are critical determinants of the nature of these complex interactions. In vitro data, strongly suggested that MSCs act as endogenous regulators of tissue inflammation with both proinflammatory and anti-inflammatory capacities depending on the biologic environment. Thus, the inflammatory environment has previously been shown to modify the fate of different stromal cell types that may alter their therapeutic functions and eventually contribute to tissue injury. Deleterious long-term adverse events secondary to the pathogenic contribution of MSCs by promoting Th17 response need to be carefully addressed when proposing future cell-based therapy. Feasible strategies are needed for monitoring the disease activity as well as profiling the inflammatory status of patients at the time of MSC infusion will obviously help optimize cell-based therapy particularly when MSCs are being used in patients with inflammatory and immune diseases. In conclusion, the emerging role of MSCs as an important therapeutic tool to treat chronic illnesses necessitates the identification of the molecular processes guiding such interactions between MSCs and T cells.

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AUTHOR CONTRIBUTIONS

All authors contributed to this study and participated in the writing and the drafting process as well as the critical review the manuscript. WF, MN and HFK did the statistical analysis and significantly contributed to the writing process. All authors read and agreed the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

REFERENCES

Angelotti, F., Parma, A., Cafaro, G., Capecchi, R., Alunno, A., & Puxeddu, I. (2017). One year in review 2017: Pathogenesis of rheumatoid arthritis. *Clinical and Experimental Rheumatology*, *35*(3), 368–378.

Bourin, P., Bunnell, B. A., Casteilla, L., Dominici, M., Katz, A. J., March, K. L., … Gimble, J. M. (2013). Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: A joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy*, *15*(6), 641–648, https://doi.org/10.1016/j.jcyt.2013.02.006

Di Battista, J. A., Shebaby, W., Kizilay, O., Hamade, E., Abou Merhi, R., Mebarek, S., … Faour, W. H. (2014). Proliferation and differentiation of human adipose-derived mesenchymal stem cells (ASCs) into osteoblastic lineage are passage dependent. *Inflammation Research*, *63*(11), 907–917. https://doi.org/10.1007/s00011-014-0764-y

Busser, H., De Bruyn, C., Urbain, F., Najar, M., Pieters, K., Raicevic, G., … Lagneaux, L. (2014). Isolation of adipose-derived stromal cells without enzymatic treatment: Expansion, phenotypical, and functional characterization. *Stem Cells and Development*, *23*(19), 2390–2400. https://doi.org/10.1089/scd.2014.0071

Chen, H., Min, X. H., Wang, Q. Y., Leung, F. W., Shi, L., Zhou, Y., … Chen, Q. K. (2015). Pre-activation of mesenchymal stem cells with TNF-alpha, IL-1beta and nitric oxide enhances its paracrine effects on radiation-induced intestinal injury. *Scientific Reports*, *5*, 8718. https://doi.org/10.1038/srep08718

Fayyad-Kazan, H., Faour, W. H., Badran, B., Lagneaux, L., & Najar, M. (2016). The immunomodulatory properties of human bone marrow-derived mesenchymal stromal cells are defined according to multiple immunobiological criteria. *Inflammation Research*, *65*(6), 501–510. https://doi.org/10.1007/s00011-016-0933-2

Feisst, V., Meidinger, S., & Locke, M. B. (2015). From bench to bedside: Use of human adipose-derived stem cells. *Stem Cells and Cloning: Advances and Applications*, *8*, 149–162. https://doi.org/10.2147/SCCAA.S64373

Frese, L., Dijkman, P. E., & Hoerstrup, S. P. (2016). Adipose tissue-derived stem cells in regenerative medicine. *Transfusion Medicine and Hemotherapy*, *43*(4), 268–274. https://doi.org/10.1159/000448180

Gao, F., Chiu, S. M., Motan, D. A., Zhang, Z., Chen, L., Ji, H. L., … Lian, Q. (2016). Mesenchymal stem cells and immunomodulation: Current status and future prospects. *Cell Death & Disease*, *7*, e2062. https://doi.org/10.1038/cddis.2015.327

Gimble, J. M., Bunnell, B. A., Frazier, T., Rowan, B., Shah, F., Thomas-Porch, C., … Wu, X. (2013). Adipose-derived stromal/stem cells: A primer. *Organogenesis*, *9*(1), 3–10. https://doi.org/10.4161/org.24279

Glenn, J. D., & Whartenby, K. A. (2014). Mesenchymal stem cells: Emerging mechanisms of immunomodulation and therapy. *World Journal of Stem Cells*, *6*(5), 526–539. https://doi.org/10.4252/wjsc.v6.i5.526

Guo, Z., Zheng, C., Chen, Z., Gu, D., Du, W., Ge, J., … Yang, R. (2009). Fetal BM-derived mesenchymal stem cells promote the expansion of human Th17 cells, but inhibit the production of Th1 cells. *European Journal of Immunology*, *39*(10), 2840–2849. https://doi.org/10.1002/eji.200839070

Ivanov, I. I., McKenzie, B. S., Zhou, L., Tadokoro, C. E., Lepelley, A., Lafaille, J. J., … Littman, D. R. (2006). The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17 + T helper cells. *Cell*, *126*(6), 1121–1133. https://doi.org/10.1016/j.cell.2006.07.035

Iwakura, Y., Ishigame, H., Saijo, S., & Nakae, S. (2011). Functional specialization of interleukin-17 family members. *Immunity*, *34*(2), 149–162. https://doi.org/10.1016/j.immuni.2011.02.012

Korn, T., Bettelli, E., Oukka, M., & Kuchroo, V. K. (2009). IL-17 and Th17 Cells. *Annual Review of Immunology*, *27*, 485–517. https://doi.org/10.1146/annurev.immunol.021908.132710

Krampera, M., Cosmi, L., Angeli, R., Pasini, A., Liotta, F., Andreini, A., … Annunziato, F. (2006). Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells.

Stem Cells, *24*(2), 386–398. https://doi.org/10.1634/stemcells.2005-0008

Kyurkchiev, D., Bochev, I., Ivanova-Todorova, E., Mourdjeva, M., Oreshkova, T., Belemezova, K., & Kyurkchiev, S. (2014). Secretion of immunoregulatory cytokines by mesenchymal stem cells. *World Journal of Stem Cells*, *6*(5), 552–570. https://doi.org/10.4252/wjsc.v6.i5.552

Maddur, M. S., Miossec, P., Kaveri, S. V., & Bayry, J. (2012). Th17 cells: Biology, pathogenesis of autoimmune and inflammatory diseases, and therapeutic strategies. *American Journal of Pathology*, *181*(1), 8–18. https://doi.org/10.1016/j.ajpath.2012.03.044

Middleton, G. W., Annels, N. E., & Pandha, H. S. (2012). Are we ready to start studies of Th17 cell manipulation as a therapy for cancer? *Cancer Immunology and Immunotherapy*, *61*(1), 1–7. https://doi.org/10.1007/s00262-011-1151-y

Mills, K. H. (2008). Induction, function and regulation of IL-17-producing T cells. *European Journal of Immunology*, *38*(10), 2636–2649. https://doi.org/10.1002/eji.200838535

Miossec, P., Korn, T., & Kuchroo, V. K. (2009). Interleukin-17 and type 17 helper T cells. *New England Journal of Medicine*, *361*(9), 888–898. https://doi.org/10.1056/NEJMra0707449

Muranski, P., & Restifo, N. P. (2013). Essentials of Th17 cell commitment and plasticity. *Blood*, *121*(13), 2402–2414. https://doi.org/10.1182/blood-2012-09-378653

Najar, M., Rouas, R., Raicevic, G., Boufker, H. I., Lewalle, P., Meuleman, N., … Lagneaux, L. (2009). Mesenchymal stromal cells promote or suppress the proliferation of T lymphocytes from cord blood and peripheral blood: The importance of low cell ratio and role of interleukin-6. *Cytotherapy*, *11*(5), 570–583. https://doi.org/10.1080/14653240903079377

Pandolfi, F., Cianci, R., Pagliari, D., Landolfi, R., & Cammarota, G. (2009). Cellular mediators of inflammation: Tregs and TH17 cells in gastrointestinal diseases. *Mediators of Inflammation*, *2009*, 132028. https://doi.org/10.1155/2009/132028

Pricola, K. L., Kuhn, N. Z., Haleem-Smith, H., Song, Y., & Tuan, R. S. (2009). Interleukin-6 maintains bone marrow-derived mesenchymal stem cell stemness by an ERK1/2-dependent mechanism. *Journal of Cellular Biochemistry*, *108*(3), 577–588. https://doi.org/10.1002/jcb.22289

Puissant, B., Barreau, C., Bourin, P., Clavel, C., Corre, J., Bousquet, C., … Blancher, A. (2005). Immunomodulatory effect of human adipose tissue-derived adult stem cells: Comparison with bone marrow mesenchymal stem cells. *British Journal of Haematology*, *129*(1), 118–129. https://doi.org/10.1111/j.1365-2141.2005.05409.x

Raicevic, G., Rouas, R., Najar, M., Stordeur, P., Id Boufker, H., Bron, D., … Lagneaux, L. (2010). Inflammation modifies the pattern and the function of Toll-like receptors expressed by human mesenchymal stromal cells. *Human Immunology*, *71*(3), 235–244. https://doi.org/. https://doi.org/10.1016/j.humimm.2009.12.005

Sheng, H., Wang, Y., Jin, Y., Zhang, Q., Zhang, Y., Wang, L., … Li, N. (2008). A critical role of IFNgamma in priming MSC-mediated suppression of T cell proliferation through up-regulation of B7-H1. *Cell Research*, *18*(8), 846–857. https://doi.org/10.1038/cr.2008.80

Di Taranto, G., Cicione, C., Visconti, G., Isgro, M. A., Barba, M., Di Stasio, E., … Lattanzi, W. (2015). Qualitative and quantitative differences of adipose-derived stromal cells from superficial and deep subcutaneous lipoaspirates: A matter of fat. *Cytotherapy*, *17*(8), 1076–1089. https://doi.org/10.1016/j.jcyt.2015.04.004

Usha Shalini, P., Vidyasagar, J. V., Kona, L. K., Ponnana, M., & Chelluri, L. K. (2017). In vitro allogeneic immune cell response to mesenchymal stromal cells derived from human adipose in patients with rheumatoid arthritis. *Cellular Immunology*, *314*, 18–25. https://doi.org/10.1016/j.cellimm.2017.01.008

Wang, W. B., Yen, M. L., Liu, K. J., Hsu, P. J., Lin, M. H., Chen, P. M., … Yen, B. L. (2015). Interleukin-25 mediates transcriptional control of PD-L1 via STAT3 in multipotent human mesenchymal stromal cells (hMSCs) to suppress Th17 responses. *Stem Cell Reports*, *5*(3), 392–404. https://doi.org/10.1016/j.stemcr.2015.07.013

Waterman, R. S., Tomchuck, S. L., Henkle, S. L., & Betancourt, A. M. (2010). A new mesenchymal stem cell (MSC) paradigm: Polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype. *PLOS One*, *5*(4), e10088. https://doi.org/10.1371/journal.pone.0010088

Zhang, L., Yuan, S., Cheng, G., & Guo, B. (2011). Type I IFN promotes IL-10 production from T cells to suppress Th17 cells and Th17-associated autoimmune inflammation. *PLOS One*, *6*(12), e28432. https://doi.org/10.1371/journal.pone.0028432

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