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Original Research Article

Ameliorative Effects of Monoclonal Antibodies (CD3 and PCNA) on the Immunomodulation of Mesenchymal Stem Cells in Male Mice

Nabil A Soliman[1]*, Heba M Saad El-Dien[2], Aya Hussin[1]

[1]*Department of Zoology, Faculty of Science, Zagazig University, Egypt*
[2]*Tissue Culture and Molecular Biology Center, Faculty of Medicine, Assuit University, Egypt*

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ABSTRACT

Mesenchymal stem cell (MSC) is being used in regenerative medicine and also for the treatment of autoimmune diseases and graft-versus-host disease. Cyclophosphamide (CTX) is a chemotherapeutic agent used for the treatment of cancer as well as several immunological diseases. The present study was designed to evaluate the therapeutic role of bone marrow-derived mesenchymal stem cells (BM-MSCs) in ameliorating the adverse effect of cyclophosphamide-induced immunohistochemical toxicity in adult male albino mice. Forty male albino mice of matched age were used for this study. They were divided into four equal groups. Group I served as the control; Group II was treated with cyclophosphamide (CTX); Group III was administered with cyclophosphamide concomitantly with MSCs; while the fourth group was used to isolate bone marrow. Blood leukocyte, splenocyte numbers, blood cell type levels, and splenocyte were determined after one month of initiation of the experiment. Two markers; the cluster of differentiation 3 (CD3) and proliferating cell nuclear antigen (PCNA) were used for immunohistochemical analysis. The results showed that CTX induced a reduction in overall proliferating cells in splenic parenchyma with clear CD3 positive cells in the periarterial sheath. MSCs induced reduction in T-cell number sparing other cells as indicated from intense cell proliferation in the splenic white and red pulp in this group of animals. The study demonstrated that MSCs induced reduction of T-cell number associated with a proliferation of other immune cells of the spleen.

Keywords: Cluster of differentiation, Cyclophosphamide, Mesenchymal stem cell, Proliferating cell nuclear antigen, T-cells.

Introduction

Mesenchymal stem cells (MSCs) are undifferentiated cells capable of dividing indefinitely with the potential to give rise to various mature specialized cell types. They were first described in 1968 by Friedenstein *et al.*[1] who from bone marrow (BM) isolated adherent, fibroblast-like clonogenic cells called colony-forming unit-fibroblasts (CFU-Fs). These cells showed a strong capacity for replication *in vitro*, could differentiate into osteoblasts, chondrocytes, adipocytes, and supported hematopoietic stroma when a single CFU-F was retransplanted *in vivo*. Following these pioneering studies, several scientists isolated and cultivated the entire population of BM stromal cells identified as cultures of MSCs.[2] However, the heterogeneity of isolation and cultivation procedures among laboratories prompted the International Society for Cellular Therapy (ISCT) to establish criteria for identifying unique populations of MSCs. In 2006, the ICST defined MSCs according to the following criteria: (i) MSCs must be purified from the BM stromal population-based on plastic adherence under standard culture conditions; (ii) MSCs must be positive for CD105, CD90, and CD73, express low levels of MHC-I, and be negative for MHC-II, CD11b, CD14, CD34, CD45, and CD31; and (iii) MSCs must differentiate *in vitro* into osteocytes, chondrocytes, and adipocytes.[3]

*Corresponding author. E mail: nabilsoliman5419@yahoo.com
Tel: 00201096380800

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Immunomodulation of MSCs may have immunosuppressive or immunostimulatory effects. The precise immunomodulatory properties of MSCs, which either up-regulate or down-regulate the immune response, are still being debated. Several factors contribute to this debate; the lack of MSCs allogeneic immune response,[4] the immunosuppressive role of soluble factors and MSCs-cell contact,[5] as well as their tumorigenic potential.[6] The absence of Antigen-Presenting cells (APCs)-associated markers may play a role in this immunosuppressive effect, at least in part. MSCs' immunomodulatory and tissue-repairing properties may aid in cell regeneration in the context of Type 1 diabetes (T1D). It is well understood that a properly functioning immune system is critical for the early detection and eradication of tumor cells. Thus, the immunomodulatory activity that MSCs may have could be incorporated into their antitumor potential. Furthermore, human MSCs do not promote the growth of allogeneic or xenogeneic lymphocytes.[7] A recent evidence, on the other hand, suggests that MSC can function as antigen-presenting cells and activate immune responses under certain conditions. The first indications of MSC's immunosuppressive nature came from studies with a human, baboon, and murine MSC, which demonstrated that MSC could suppress thymus lymphocyte (T-lymphocyte) activation and proliferation *in vitro*.[8] Several studies have shown that MSC inhibits T-cell proliferation by arresting T-cells in the G0/G1 phase.[9] Benvenuto *et al.*[10] have made significant contributions in this regard. MSC inhibits T-cell proliferation while also promoting the survival of T-cells that have been overstimulated via the T-cell receptor and are committed to CD95-CD95 ligand-dependent activation-induced cell death. MSC also inhibits interferon (IFN) production while increasing interleukin-4 production by T-helper 2 cells. This indicates that T-cells have shifted from a pro-inflammatory to an anti-inflammatory state.[11]

Glennie *et al.*[12] discussed the pathways involved in the inhibition of T-cell proliferation. The effect is attributed to MSC-induced inhibition of cyclin D2 and up-regulation of p27kip1, which causes T-lymphocytes to be arrested in the G1 phase. The mechanisms underlying these effects are unknown, but they are most likely mediated by direct cell-cell contacts as well as soluble factors such as nitric oxide and indoleamine 2,3-dioxygenase, which are released by MSC after being activated by IFN produced by target cells.[13] Other soluble factors that MSC produce constitutively include transforming growth factor-1, hepatocyte growth factor, prostaglandin E2 (PGE2), IL-10, haemoxygenase-1, IL-6, and human leukocyte antigen-G5 (HLA-G5).[14] The present study was conducted to evaluate the therapeutic role of bone marrow-derived mesenchymal stem cells (BM-MSCs) in ameliorating the adverse effect of CTX-induced immunohistochemical toxicity in adult male albino mice.

Materials and Methods

Sources of drugs and chemicals

Trypan blue/0.4% PBS (phosphate buffer solution) and dimethyl sulfoxide (DMSO) used for this study were purchased from Sigma, Aldrich, USA. Also, 1% Penicillin, Streptomycin B mixture (10 IU/2.5 µg, 100 mL), 0.25% trypsin/ethylene diamine tetraacetic acid (EDTA) high glucose DMEM, 4.5 g/L glucose with L-glutamine, Lymphocyte separation medium 1.077, 10% fetal bovine serum (FBS, Phosphate buffer saline (PBS), were all obtained from Lonza Bioproducts, Belgium.

Source and acclimatization of experimental animals

This study was conducted in the Tissue Culture and Molecular Biology Center (Stem Cell Unit), Faculty of Medicine, Assiut University in collaboration with the Physiology Division, Department of Zoology, Faculty of Science, Zagazig University. A total number of 40 male albino mice of matched age and weight, used for the study were obtained from the animal house of the Center of Stem Cell Unit, Faculty of Medicine, Assiut University. The mice were kept in an environmentally controlled room (23–25°C, 40–70% relative humidity, and 12 h light/dark cycle) with free access to standard mice feed and water. Mice were maintained on a stock diet and kept under appropriate conditions of housing and handling.

Ethical approval

The experimental animals used in the present study and experimental protocol were approved by the Research Ethics Committee of Zagazig University with an Approval No. of ZU-IACUC/1/F/31/2019. All laboratory procedures were carried out following the Guide for the Care and Use of Laboratory Animals.

Experimental design

A total number of 40 healthy male mice were divided randomly into 4 groups. The first group consisted of 10 healthy male albino mice which served as the normal control group. The second group consisted of 10 male albino mice which received cyclophosphamide at a dose of 50 mg/kg body weight. The third group consisted of 10 male albino mice treated with cyclophosphamide at a dose of 50 mg/kg, then after 15 days of injection, a dose of stem cells containing one million cells/kg was injected intraperitoneally (i.p.). The fourth group (10 mice) served for bone marrow isolation. The animals were weighed again one month after the experiment began, dosages were adjusted based on the body weight and the treatments were administered for 30 days.

Immunohistochemical analysis

Immunohistochemical analysis was performed using the streptavidin–peroxidase method (UltraVision Plus Large Volume Detection System Anti-Polyvalent, HRP immunostaining Kit, Thermo Scientific, UK). To reduce nonspecific background staining due to endogenous peroxidase, cultured cells were fixed in ice-cold methanol comprising 0.3% hydrogen peroxide (Carlo Erba Reactions, Val-De-ReuilCedex Pa Des Portes, France) for 15 min and allowed to dry. After additional PBS washes, cells were incubated with Ultra V Block for 5 min at room temperature. The following day, cells were incubated with biotinylated secondary antibodies for 15 min at room temperature. Incubations were followed by streptavidin–peroxidase treatment for 15 min at room temperature and detection of signals was completed by the use of an AEC kit (Zymed Laboratories, UK).

Statistical analysis

Data were analyzed using IBM SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA) and NCSS 11 for Windows (NCSS LCC., Kaysville, UT, USA). Quantitative data were expressed as mean ± standard deviation (SD) while qualitative data were presented as frequency and percentage. Significance was accepted at $P< 0.05$.

Results and Discussion

The results of the haematological assessment after 30 days post-CP treatment and with or without MSCs revealed that there was a significant difference among the three treatment groups (Table 1). Also, there was a significant ($P<0.05$) increase in all the haematological parameters in Group III, and a significant ($P<0.05$) decrease in all the haematological parameters in group II after 1 month, while the changes among the control group were not significant. The current study found a statistically significant ($P<0.05$) decrease in haematological parameters in the cyclophosphamide (CTX)-treated group after one month of treatment compared to the control group (Table 1), which is consistent with previous research.[15] This observation is in line with the findings of Yeh *et al.*,[16] who discovered that CTX treatment can cause nausea, vomiting, low blood cell counts, including red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs), and myelosuppression. Furthermore, Botnick *et al.*[17] discovered that a high dose of CTX causes acute bone marrow depression, which can lead to the death of hematopoietic progenitors. Also, CTX has been reported to act as an immunosuppressive agent.[18] CTX treatment, according to Petrucci *et al.*,[19] reduces the number of RBCs, WBCs, and PLTs.

It has also been reported that CTX can cause myelodysplastic syndromes or leukemia before transplantation of hematopoietic stem cells (HSCs) from various donors, as well as an effective treatment.[20] CTX is particularly toxic to rapidly proliferating tissues such as the hematopoietic system, gastrointestinal epithelia, hair follicles, and genital glands. In a study conducted by De Jonge *et al.*,[21] they reported that human WBC counts began to decline on day 6 and reached a nadir between 9 and 12 days. Wang *et al.*,[22] were able to show that half of the CD1 mice in their research developed leukopenia after receiving intraperitoneal injections of CTX (150 mg/kg) three days before and one day after the bacterial challenge. On day 4, a single intraperitoneal injection of 4 mg/kg CTX resulted in a significant decrease in peripheral blood stem cells (PBSCs) in C57BL/6 mice.

In the current study, there was a significant increase in the haematological parameters in the group that received cyclophosphamide along with MSCs compared to the group that received only cyclophosphamide. This observation indicated that MSCs are safe and effective when used in allogeneic BMT for the treatment of haematological disorders. Cytotoxic drugs were used to induce BM ablation in mice, and BMT was performed with or without MSC. On the other hand, the present findings suggest that MSC promotes BM engraftment to increase mouse survival. As a result, MSC efficiently promotes BM reconstitution, which could be considered a novel therapeutic strategy to improve the efficacy of hematopoietic stem cell transplantation (HSCT). This was consistent with the findings of Kim *et al.*,[23] who made the same observations. MSCs were isolated from rat bone marrow with 80-90% confluence in the current study. Using the immunophenotyping characterization Cd31, 90, 45, the magnified MSCs appeared as spindle fibroblast-like shapes (Figures 1A-C). In agreement with the findings in this study, Karaoz *et al.*[24] discovered that MSCs attached sparingly to culture flasks and displayed a fibroblast-like, spindle-shaped morphology during the first day of incubation. Furthermore, Ponnaiyan *et al.*[25] discovered that morphologically similar fibroblast-like cells could be easily isolated from both fetal and adult sources, including the umbilical cord, adipose tissue, skin, dental pulp, and liver.

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Table 1: Changes in blood elements in the treatment groups

Variables	Group I N=10	Group II N=10	Group III N=10	F test	P-value	LSD for difference in between groups
Haemoglobin (mg/dl)	12.5±0.67^{NS}	12.3± 0.8**	12.4 ± 0.88**	0.13	0.88 NS	
Haemoglobin after 1 month	12.2±0.69^{NS}	9.6± 0.58**	13.2± 0.75**	76.5	<0.001 HS	P1<0.001 P2=0.004 P3<0.001
Platelets	341.7±68.7$^{\#}$	335.5±73.2$^{\#\#}$	362.7±44.98$^{\#\#}$	0.51	0.61 NS	
Platelets after 1 month (L)	332.5±72.1$^{\#}$	166.8±51.1$^{\#\#}$	538 ±75.8$^{\#\#}$	76.5	<0.001 HS	P1<0.001 P2<0.001 P3<0.001
WBCs	8.26 ± 1.5^{N}	7.83 ± 1.8^{N}	7.79 ± 1.7^{N}	0.24	0.79 NS	
WBCs after 1 month	7.86 ± 1.7^{N}	4.57 ± 1.11**	9.43 ± 1.7**	26.3	<0.001 HS	P1<0.001 P2=0.03 P3<0.001
Lymphocytes	3.31±0.62^{N}	3.25 ± 0.81$^{\#\#}$	3.22 ± 0.71$^{\#\#}$	0.04	0.96 NS	
Lymphocytes after 1 month	3.31±0.63^{N}	1.93 ± 0.81$^{\#\#}$	3.91 ± 0.3$^{\#\#}$	27.1	<0.001 HS	P1<0.001 P2=0.04 P3<0.001
Neutrophil	5.22 ± 1.2^{N}	5.37 ± 1.14$^{¥¥}$	5.53 ± 0.96$^{¥¥}$	0.19	0.83 NS	
Neutrophil after 1 m.	5.25±1.16^{N}	2.33 ± 0.12$^{¥¥}$	6.34 ± 0.62$^{¥¥}$	43.5	<0.001 HS	P1<0.001 P2=0.02 P3<0.001

*The values in the upper row for each parameter represent the baseline values.
All data are expressed as Mean ± SD

- The P-value for change in Hb within groups by paired t-test within each group NS means non-significant ** means highly significant P1<0.001
- The P-value for change in platelets within groups by paired t-test within each group # means significant P1<0.05 ## means highly significant P<0.001
- The P-value for change between groups of WBCs N means non-significant
- The P-value for change in WBCs within groups^{N} means non-significant P>0.05 ** means highly significant P<0.001
- The P-value for change in lymph within groups$^{\#}$ groups^{N} means non-significant P>0.05 $^{\#\#}$ means highly significant P<0.001
- The P-value for change in neutrophil within groups^{N} means non-significant P>0.05 $^{¥¥}$ means highly significant P<0.001
- P1: for the difference between group I and II
- P2: for the difference between group I and III
- P3: for the difference between group II and I

Friedenstein *et al.*[26] described the isolation of clonogenic, proliferating fibroblastic MSCs from rat bone marrow for the first time. They demonstrated that stromal cells derived from CFU-Fs can serve as feeder layers for the culture of hematopoietic stem cells (HSCs) and can differentiate into osteocytes, chondrocytes, and adipocytes.
According to the International Society for Cellular Therapy's criteria, these cells have a specific surface-molecule phenotype, being positive for CD105, CD73, and CD29 expression and negative for CD31, CD34, CD45, CD14, and human leukocyte antigen (HLA)-DR expression (ISCT).[27] MSCs, according to Karaoz *et al.*,[24] are multipotent cells that can differentiate into multiple lineages both *in vitro* and *in vivo*. MCSs can be isolated from bone marrow and expanded or manipulated in cultures relatively easily. These characteristics allow for an autologous cell source, which in some cases avoids host immune response issues, and makes them an attractive candidate for potential therapeutic applications such as cellular and gene therapies, tissue engineering, small-animal model-based vascular biology studies, *in vivo* imaging techniques, and other preclinical investigations. Rat bone marrow-derived MSCs (RBM-MSCs) isolated in various laboratories around the world share at least two characteristics: (i) rBM-MSCs can grow for a long time (a process known as long-term self-renewal); and (ii) rBM-MSCs can differentiate into mesodermal lineage cells such as osteoblasts,[28] chondrocytes,[29] muscle cells,[30] adipocytes,[28] and tenocytes in response to appropriate stimuli.[31] The expression of cell-surface markers such as CD29, CD44, CD90, and CD106 is the general strategy for identifying *in vitro* cultivated rBM-MSCs.[32] The expression of surface antigens, on the other hand, agreed with previous reports on murine MSCs and indicated that the cells used in the current study had the characteristics of MSCs reported elsewhere.[32]

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The findings from this study suggest that due to the high proliferation ability of BM-MSCs, they are a promising source for future clinical use. BM-MSCs have a superior capacity for osteogenic differentiation and thus may be a good cell source for tissue engineering. Many of these antigen expressions explain why these cells can readily differentiate into functional multiple lineages both *in vitro* and *in vivo*. More so, cellular characteristics of rBM-MSCs as determined by transmission electron microscopy revealed more developed and metabolically active cells. The presence of rough endoplasmic reticulum (rER), closely packed cristae containing mitochondria, and large peripheral collections of unbound glycogen are their distinguishing features. These observations are consistent with the findings of Karaoz *et al.*[24]

Mesenchymal stem cells are an intriguing component of the microenvironment in the bone marrow and other tissues, and they were thought to play a primarily supportive role for many years. However, the focus of MSC research over the last decade has shifted dramatically, first to their potential role in tissue regeneration via broad, multi-lineage differentiation potential, and more recently, to a better understanding of their immunomodulatory properties.[33] On two counts, a better understanding of the mechanisms underlying MSC immunomodulatory effects is critical. First, it may reveal a previously unknown arm of the immune system, and second, it has significant therapeutic implications. MSCs have the potential to treat immune suppression effectively.[34]

In the present study, the immunohistochemical reaction of cyclophosphamide-treated animals was examined using a PCNA monoclonal antibody. Few positive proliferating cells were found in the red pulp (No observed positive T-cells in a periarterial lymphatic sheath of the spleen). Magnified images revealed a few proliferating cells in the spleen's red pulp (Figures 2A&B). On the other hand, immunohistochemical reaction using the CD3 monoclonal antibody of cyclophosphamide treated animals (Figures 3A-D) showed positive T-cells in a periarterial lymphatic sheath of the spleen (Figures 3A&B). When these images were magnified (Figures C [X40]; D[X400], respectively), the great density of the T-cells in PALS and marginal zone of the spleen were revealed.

MSCs, according to Castro-Manrreza *et al.*,[35] play an important role in regulating the inflammatory microenvironment and interacting with immune cells such as T-cells, B-cells, natural killer (NK)-cells, and dendritic cells (DCs). This was supported by Ren *et al.*[36] who, in this issue, makes a timely and significant contribution to the ongoing debate about how MSCs suppress T-cells, allowing a clearer picture of the mechanism to emerge. Previous research had shown that MSCs inhibit the proliferation of T-cells induced by alloantigens and nonspecific mitogens and that this inhibition is not genetically restricted.[37]

The immunohistochemical reaction using PCNA monoclonal antibody of MSCs treated animals in Figures 4A and 4B (X400) indicated that numerous positive proliferating cells in the red pulp (No observed positive T-cells in a periarterial lymphatic sheath of the spleen). On the other hand, immunohistochemical reactions using the CD3 monoclonal antibody on MSCs-treated animals revealed reduced positive T-cells in the spleen's periarterial lymphatic sheath (Figures 5A). When this image was magnified (Figures B[X40]; C[X400]), the low density of T-cells in the PALS and splenic marginal zone were shown.

Furthermore, several studies have shown that the suppression is variable in transwell experiments, implying that a soluble factor(s) is involved, though other researchers claim that MSC-T cell contact is required.[38] Other *in vitro* experiments yielded conflicting results: one group suggested TGF-b and hepatocyte growth factor,[39] another group suggested interleukin-2 and interleukin-10,[40] and others suggested prostaglandin E_2.[41] Another possibility is that interferon-γ (IFN-γ) induces MSC-derived indoleamine 2,3-dioxygenase (IDO), which catabolizes tryptophan, suppressing T-cell proliferation and causing apoptosis in activated T-cells.[33]

Based on the observation that BM-MSCs strongly suppress T-lymphocyte proliferation due to the production of soluble factors, Zhou *et al.*[42] discovered that the secretion of bioactive factors, such as chemokines and cytokines, contributes to the broad effects of MSCs on cells of the innate and adaptive immune systems. Sato *et al.*[43] demonstrated that the suppressive effect is triggered by the T-cell receptor complex. Their findings showed that MSCs produce nitric oxide (NO) in a dose-dependent manner in response to CD4+ or CD8+ T-cells and that NO is involved in the suppression of Stat5 phosphorylation in T-cells, resulting in a decrease in T-cell proliferation. The researchers also discovered that inhibiting prostaglandin synthase or nitric oxide synthase (NOS) restored T-cell proliferation, whereas inhibiting indoleamine (IDO) or neutralizing TGF-*β* did not.

Ren *et al.*[36] confirmed Sato's findings and expanded on them to provide a coherent mechanism. They used an appealing combination of traditional immunoassays, inhibitor studies, and MSCs derived from knockout mice that targeted either the IFN-γ receptor or inducible NOS. They discovered that IFN-γ combined with any of the proinflammatory cytokines; Tumor necrosis factor-alpha TNF-α, IL-1α, or IL1β causes immunosuppression. They also demonstrated that such combinations cause the release of NO and chemokines from MSCs independently. Chemokines attract immune cells to the area, including T-cells that express the chemokine receptor CXCR3.

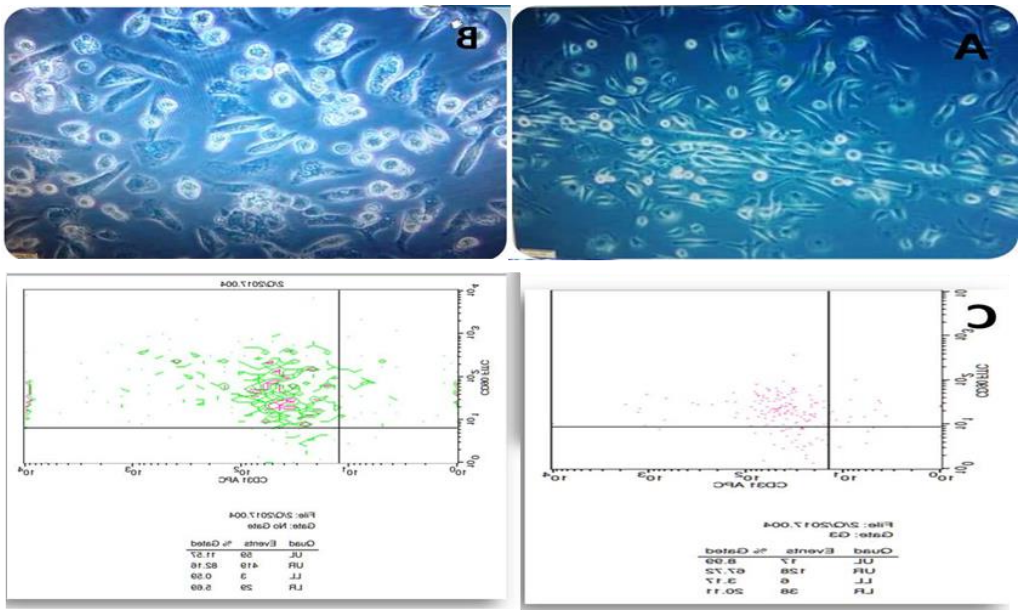

Figure 1: Mesenchymal stem cells (MSCs).
A: MSCs isolated from rat bone marrow, with 80-90% confluence; **B:** Magnified MSCs appear spindle fibroblast like shape; **C:** The immunophenotyping characterization of MSCs using Cd31, 90, 45.

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The NO suppresses the proliferation of neighboring T-cells, resulting in an apparent negative feedback loop that dampens an overzealous immune response to inflammation, injury, or an all-out attack. In contrast to previous studies, the authors were unable to abolish immune suppression with indomethacin, a prostaglandin synthase inhibitor, but confirmed previous findings that an IDO inhibitor or neutralizing antibodies against IL-10 or Transforming growth factor-beta (TGF-β) does not affect suppression. Interestingly, they were unable to demonstrate the suppressive effect of NO in transwell experiments and attributed this observation to the rapidly diminishing concentration of the active form from the cell source. Moreover, MSC-derived paracrine factors influence T-cells by regulating innate immune cells such as macrophages and DCs, in addition to their direct effects on T-cells. Co-stimulatory ligands must interact with the T-cell receptor (TCR) on T-cells for T-cells to be activated. Thus, soluble factors produced by MSCs can influence the expression of co-stimulatory ligands by APCs (antigen-presenting cells), thereby modulating T-cells. MSCs, for example, influence macrophage polarisation, thereby controlling T-cell differentiation and, ultimately, exerting an immunomodulatory function.[44]

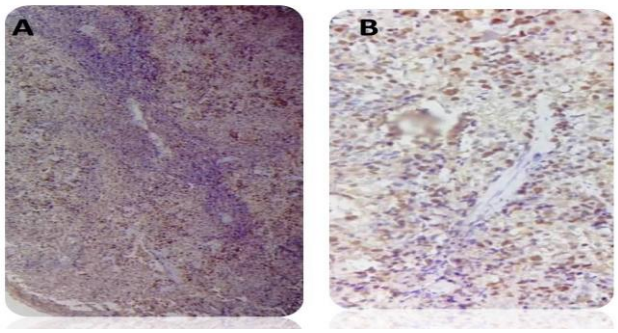

Figure 2: Immunohistochemical reaction using PCNA monoclonal antibody of cyclophosphamide-treated animals. Few positive proliferating cells in the red pulp (No observed positive T-cells in a periarterial lymphatic sheath of the spleen). A: Magnified previous images (X40); B: Few proliferating cells in the red pulp of the spleen (X400)

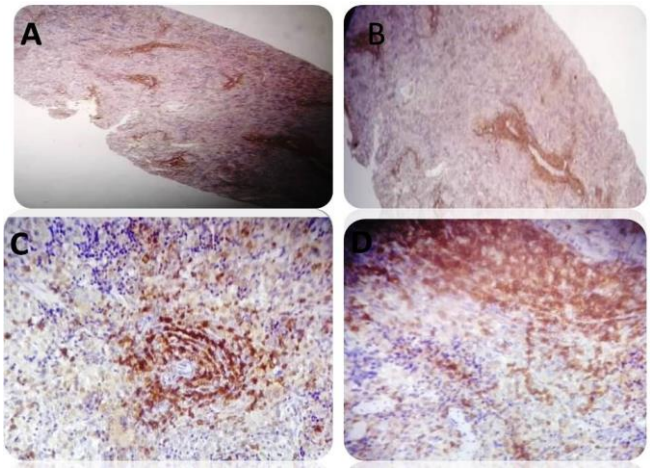

Figure 3: Immunohistochemical reaction using Cd3 monoclonal antibody of cyclophosphamide-treated animals. A&B: Positive T-cells in a periarterial lymphatic sheath of the spleen; C&D: Magnified images of A&B, respectively showing the great density of T-cells in PALS and marginal zone of the spleen (C=X40, D=X400).

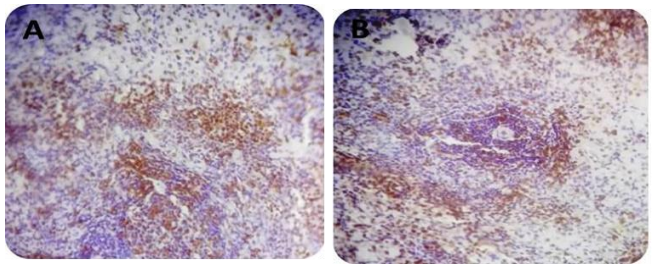

Figure 4: Immunohistochemical reaction using PCNA monoclonal antibody of MSCs-treated animals. A&B: Numerous positive proliferating cells in the red pulp (No observed positive T-cells in a periarterial lymphatic sheath of the spleen (X400).

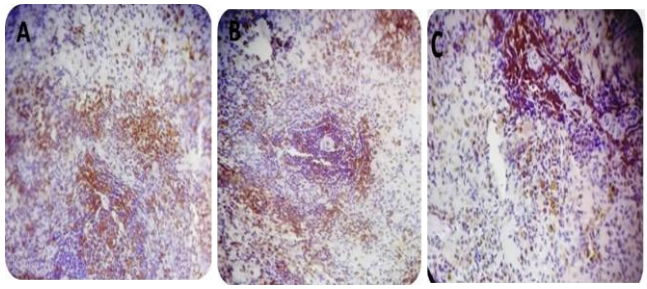

Figure 5: Immunohistochemical reaction using the CD3 monoclonal antibody of MSCs-treated animals. A: Reduced positive T-cells in a periarterial lymphatic sheath of the spleen; B&C: Magnified image of A, showing the low density of T-cells in PALS and marginal zone of the spleen (B=X40, C=X400).

Furthermore, Zhao *et al.*[45] discovered that MSCs' large quantity expansion and multi-potential differentiation make them an ideal candidate for stem cell-based therapy and gene carriers. Another intriguing aspect of MSCs is their ability to evade immune recognition and inhibit immune responses. As a result, MSCs will be a very promising immunomodulatory cell therapy tool in immune-mediated diseases. MSCs have also been shown to have extensive immunoregulatory capabilities, influencing both adaptive and innate immune responses. In a non-MHC restricted manner, MSCs inhibit immune cell proliferation and maturation and suppress immune reactions *in vitro* and *in vivo*.[46] As a result, MSCs are considered hypoimmunogenic, with low levels of HLA class I expression, no expression of HLA class II, and no expression of co-stimulatory molecules such as CD40, CD80, and CD86. MSCs have the potential to have broad immunomodulatory effects on cells of both the innate and adaptive immune systems. *Ex-vivo* expanded MSCs have also been shown to suppress the activity of a wide variety of immune cells, including T-cells, natural killer T (NKT)-cells, dendritic cells (DCs), B-cells, neutrophils, monocytes, and macrophages.[47] Cell proliferation and cytokine secretion are the two most important aspects of the T-cell response. The most significant effect of MSCs is to inhibit T-cell proliferation. *In vitro*, MSCs can suppress T-lymphocyte proliferation induced by mitogens and alloantigens, as well as T-cell activation induced by CD3 and CD28 antibodies.[48] MSC-induced suppression of T-cell proliferation has no immunological limitations, with similar suppressive effects observed with cells that were autologous or allogeneic to responder cells.[49] MSCs also modulate immune responses by inducing regulatory T-cells, which are essential for immune homeostasis and self-tolerance. MSCs have been shown to stimulate the development of regulatory T-cells, which are responsible for the inhibition of allogeneic lymphocyte proliferation.[50]

Furthermore, mitogen-stimulated peripheral blood mononuclear cell (PBMC) cultures in the presence of MSCs showed an increase in the population of regulatory T (Treg)-cells.[49]
Treg-cells exposed to MSCs are more immunosuppressive than Tregs not exposed to MSCs. Their findings suggested that interactions between the programmed cell death 1 receptor and the B7-H1 receptor, as well as IL-10, may be responsible for the enhanced suppressive capability of MSC-exposed regulatory T-cells.[50] However, depletion of regulatory T-cells did not affect MSC-mediated suppression of T-cell proliferation, and MSCs physically prevent T-cells from interacting with antigen-presenting cells (APCs) in a non-cognate manner. Human adipocyte-MSCs are activated in a variety of ways. When stimulated with mouse splenic T-cell culture supernatant, they performed better than when stimulated with human peripheral blood mononuclear cell (PBMC) supernatant. They did, however, have an anti-proliferative effect on mouse splenic T-cells *in vitro*, primarily through the expression of Cyclooxygenase-2 (COX-2).[51] Although the precise mechanism underlying MSCs' immunosuppressive effects is still unknown, most evidence suggests that soluble factors are involved. Prostaglandin E2 (PGE2),[52] indoleamine 2,3-dioxygenase (IDO),[53] hepatocyte growth factor (HGF),[54] and transforming growth factor (TGF)-b 1[55] are among these factors. Furthermore, it is well known that IFN-γ plays a significant role in the enhancement of MSC suppressive activity. Furthermore, there is growing evidence that MSCs inhibit the proliferation and/or functions of CD4 Th1 and Th17 cells, CD8 T cells, and natural killer cells primarily through the secretion of soluble factors such as TGFb1 and HGF.[56] MSCs also play an important role in the activation of cytotoxic CD8 T-cells against intracellular pathogens. According to Schurch *et al*.,[57] IFN-γ can promote the release of hematopoietic cytokines such as IL-6 from MSCs, which reduces the expression of the transcription factors Runx-1 and Cebpa in early hematopoietic progenitor cells, increases myeloid differentiation, and triggers the temporary activation of emergency myelopoiesis to promote infection clearance.

Conclusion

The present study demonstrated that MSCs induced reduced T-cell number associated with a proliferation of other immune cells of the spleen. MSCs have become a subject of clinical research interest due to their easy of isolation. In addition, MSCs are safe and well-tolerated for use in cell therapy, which provides a striking candidate for degenerative diseases and immune-mediated diseases. Expanding our understanding of the molecular mechanisms governing the immunomodulatory properties of MSCs will enable us to greatly improve their clinical efficacy. Most importantly, well-designed, randomized, and controlled clinical trials should be considered for a better understanding of the underlying biology and utilizing MSCs therapy for immune-mediated disease in the future.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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