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Biological and Immunological Properties of Mesenchymal Stromal Cells Derived From Bone Marrow in Childhood Acute Myeloid Leukemia

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Abstract

Objective: Pediatric acute myeloid leukemia (AML) is a common form of pediatric leukemia and is characterized by the accumulation of abnormal white blood cells, called blasts, in the bone marrow (BM). The aim of this study was to understand the BM microenvironment by studying the biological and immunological properties of BM-derived mesenchymal stromal cells (MSCs) and mononuclear cells (MNCs) to elucidate the potential role of phytohaemagglutinin in cell viability.

Materials and Methods: BM and peripheral blood samples were obtained from seven pediatric AML patients and seven donors. BM-MSCs and MNCs were isolated and characterized. Population doubling (PD) values, adipogenic and osteogenic differentiation capacity, cell viability, phytohemagglutinin (PHA) assay, and flow cytometry were performed.

Results: Mononucleated cells from peripheral blood of AML patients and donors and T-cell activation markers ($CD3^+CD69^+$, $CD4^+C25^+$, $CD3^+HLA\text{-}DR^+$) were measured by flow cytometry (χ^2=2.184; p=0.823). BM-MSCs were co-cultured with MNCs, and PD values for AML patients were similar to those of donors (z=1.074; p=0.394). It was statistically significant when healthy MNC and healthy MNC PHA(+) groups were compared (p=0.015). When healthy MNC PHA(+) and healthy MSC+AML MNC PHA(+) groups were compared, it was found to be statistically significant (p=0.014).

Conclusion: This interaction is also not unidirectional. This interaction serves as a marker for understanding the immunological effects of AML.

Keywords Pediatric AML · BM-MSCs · PHA · Cell viability · MNCs

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INTRODUCTION

The formation of aberrant myeloid progenitor cells (myeloblasts) in the bone marrow (BM) is a characteristic of acute myeloid leukemia (AML), an aggressive hematologic malignancy that frequently invades extramedullary tissues like the liver and spleen as well as the bloodstream (1, 2). Although AML is very rare in children, it is associated with a disproportionately high mortality rate (3). Isolated from extramedullary tissues, mesenchymal stromal cells (MSCs) can be used in tissue regeneration cell therapies, serving as a reserve to replace damaged and senescent cells (4). The multipotent and self-renewing nature of MSCs makes them promising for use in cell treatment, tissue repair, and regenerative medicine research (5). Within the bone marrow (BM) niche, MSCs provide support for hematopoietic progenitor and stem cells (HSPCs) (6). MSCs are extremely rare in the BM and account for less than 0.01% of all mononucleated cells (7).

MSCs remain quiet under normal circumstances. They are thought to be attracted to injured sites to restore or repair damaged tissues. They are thought to be activated and to divide symmetrically or asymmetrically upon activation by biological signals, such as those resulting from tissue injuries. MSCs have been used in a growing number of clinical indications because of their strong reparative potential, particularly for bone deformities and cartilage loss (7).

By attaching itself to the T cell receptor (TCR)CD3 complex, phytohaemagglutinin (PHA) imitates every intracellular activation event caused by anti-CD3 antibodies. Red kidney beans are a source of PHA, a lectin that attaches itself to T cell membranes to promote metabolic processes and cell proliferation (8). Four hours after cell activation, the T, B, and natural killer cell surfaces show the earliest activation marker, CD69, which is linked to the transcription of tumor necrosis factor-alpha (TNF-α) and interleukin-2 (IL-2). Increased expression of the IL-2 receptor's CD25 component is observed 12 to 24 hours following cell activation. Sixty to eighty hours later, T cells initiate to express HLA-DR (9).

The aim of this study was to understand the relationship between MSCs and the BM microenvironment, as well as the viability of the cells in pediatric AML by examining the immunological characteristics of these cells derived from the BM and to shed light on potential roles in the pathogenesis of AML.

MATERIALS AND METHODS

Research Methodology and Ethics

Seven healthy donors who were matched with 7 newly diagnosed pediatric AML patients were included in this study. The Ankara Children's Health Diseases Hematology Oncology Training and Research Hospital's clinical research ethics committee approved the haematopoietic stem cell transplantation (HSCT) protocol, and all participants provided informed consent (10, 11) (ID:2014062). At the time of diagnosis, MSCs were isolated from the BM and cultivated *ex vivo* for testing.

BM Collection

BM samples from pediatric AML patients and donors were extracted, frozen, and centrifuged under general anesthesia. Plasma was collected and stored at -80°C.

Isolation, Freezing, and Thawing of Mononuclear Cells (MNCs) Derived from BM

MNC samples were diluted with phosphate buffered saline (PBS) (Biochrom, Germany) and Biocoll (1.077 g/mL) separating solution (Biochrom, Germany) (1:1), frozen in Dulbecco's Modified Eagle Medium with low glucose (DMEM-LG) (Biochrom, Germany), thawed, centrifuged, and reconstituted in DMEM-LG, FBS, and penicillin-streptomycin (Biochrom, Germany). Cells were seeded in 75 cm^2 plastic flasks, maintained in a humidified atmosphere with 5% CO_2 at 37°C, and then seeded in a Galaxy 170R incubator (Eppendorf Company, Hamburg, Germany).

Cell Culture of BM-Derived MSCs

The cell culture medium was changed every 3 days, non-adherent cells were removed after 72 h, and adherent cells (Passage 2) were trypsinized at 37°C for 5 minutes after 70%-80% confluence.

Characterization and Differentiation Capacity of MSCs

Flow cytometry was also used to examine MSC cell surface markers. CD45-Alexa Fluor® 488 (BioLegend, cat. no. 304019), CD34-FITC (BioLegend, cat. no. 343604), CD90-PE (BioLegend, cat. no. 328110), CD73-APC (BioLegend, cat. no. 344006), and CD105-PE/Cy7 (BioLegend, cat. no. 323218) were used to stain the cells. The cells were then examined using the Navios-Beckman Coulter flow cytometer and Navios Software v1.2. Positive and negative antibody staining was also performed (BD Biosciences, Piscataway, NJ, USA). Flow cytometry was used to surface phenotype MSCs.

The differentiation potential of MSCs into adipogenic and osteogenic lineages was assessed *in vitro* using osteogenic and adipogenic supplemental supplements (Stemcell Technologies, Vancouver, Canada). On day 21, the differentiation of MSCs into these cells was confirmed using alizarin red and oil red O staining.

Proliferative MSC Assay

The proliferative capacity of MSCs was assessed using trypsinization, cell growth detection, and hematoxylin and eosin staining. The number of PDs from passage P1 to P2 was calculated using $\log_{10}(N)/\log_{10}(2)$ indicating harvested/sown cells (12, 13).

Coculture and PHA Activation Tests

MNCs from peripheral blood samples were plated on T-25 flasks in DMEM supplemented with FBS and penicillin-streptomycin for 72 hours to activate lymphocytes, with a density of $3x10^5$ cells/cm² with a density of 10 mL PHA/$1x10^6$ cells in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a humidified environment with 5% CO_2 for 72 hours in order to activate the lymphocytes. PHA-activated MNCs-MSCs were co-cultured with allogeneic human MSCs in a study, comparing control, newly isolated MNCs, PHA-activated MNCs-MSCs, and PHA non-activated MNCs-MSCs, for immunological tests. 30,000 MNCs were added to each well at a 10:1 ratio, and the cells were centrifuged and cleaned after four days. The quantity and viability of the cells were determined.

Flow Cytometry Analysis

CD45-Alexa Fluor® 488 (BioLegend, cat. no. 304019), CD34-FITC (BioLegend, cat. no. 343604), CD90-PE (BioLegend, cat. no. 328110), CD73-APC (BioLegend, cat. no. 344006), and CD105-PE/Cy7 (BioLegend, cat. no. 323218) cell surface markers were analysed for the MSCs. CD3, CD4, CD25, CD69, and HLA-DR were analyzed by flow cytometry (Beckman Coulter, Brea, CA, USA), and MNCs were extracted using Kaluza version 1.2 software.

Statistical Analyses

Statistical significance was assessed using Student's t-test and the Mann–Whitney U test $p<0.05$ was considered statistically significant. IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

Table 1. Demographic features of pediatric AML patients and donors

Pediatric AML (n=7)		**Healthy Donor (n=7)**	
Age, median (range)	13 (2-17)	Age, median (range)	10 (3-51)
Gender		Gender	
Male	3	Male	5
Female	4	Female	2
AML immunophenotyping		Total number of healthy MNC donors	1
AML	5	Median age	31
M2	0	Gender	
M4	1	Male	0
MDS	1	Female	1

Abbreviations: AML: Acute myeloid leukemia; MDS: Myelodysplastic syndrome; MNC: Mononuclear cells; n=number

Table 2. BM-derived MSC expression of CD Markers

Marker	**Donor 1**	**Donor 2**	**Donor 3**
CD90	100	99.4	99.5
CD73	90.4	88.2	93.4
CD105	95.6	89.8	90.1
CD34	0.1	0.2	0.1
CD45	0.2	0.3	0.1

Abbreviations: BM: Bone marrow; MSC: Mesenchymal stromal cells. Data are presented as percentages.

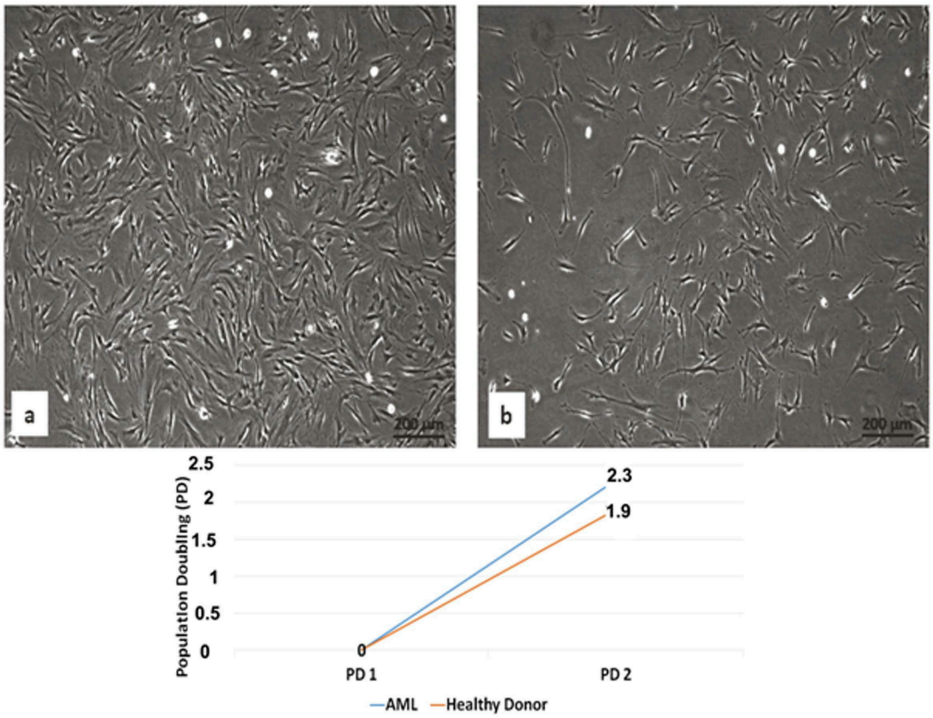
RESULTS

Demographic Features of AML Patients and Donors

In this study, the demographic features of AML and donors are presented in Table 1. The median age ranged from 2-17 years in the AML group and 3–51 years in the healthy group. The AML group had 4 females and 3 males, whereas the healthy group had 5 females and 2 males (Table 1).

Proliferation and Characterization of AML-MSCs and HD-MSCs

BM-MSCs were plated, treated with trypsin-EDTA, harvested, and measured by haemogram. Population doubling (PD) values of AML patients and healthy donors were calculated using the $\log_{10}(N)/\log_{10}(2)$ formula. AML patients had similar PD2 values to healthy donors ($z=1.074$; $p=0.394$). The median values for PD1 and PD2 were similar between the groups (Figure 1). After passage 2 of the BM-MSCs, trypan blue dye was used to examine the percentages of cellular viability. Both types of MSCs were used in the tests. The immunophenotyping study revealed that both types of MSCs had been carried for $CD34^-$, $CD3^-$, $CD4^-$, $HLA\text{-}DR^-$, $CD90^+$, $CD73^+$, $CD44^+$, $CD49e^+$ and $HLA\ ABC^+$. Differentiation tests were used to verify the ability of both the study and control groups of MSCs to differentiate into



	AML		Control			
	Median (min-max)	Mean ± SD	Median (min-max)	Mean ± SD	z	p value
Population doubling value (PD)	2.0 (2.0-3.0)	2.3 ± 0.4	2.0 (1.0-3.6)	1.9 ± 0.9	1.074	0.394

Figure 1. Cell morphology images of MSCs. **a:** BM-MSCs of AML patient; **b:** BM-MSCs of donors (Olympus CKX41-40X). Logaritmic population doubling (PD) values of BM-MSCs derived from AML patients and donors. The proliferation capacity of AML-MSCs compared with HD-MSCs. Calculated cumulative PD (PDs) from P1 to P2 of MSCs isolated from HDs and from AML patients. Statistical significance is $p<0.05$.

adipogenic and osteogenic tissues (Figure 2). The expression levels of CD markers were analyzed in BM-derived MSCs (Table 2).

Cell-viability

The statistical values of the healthy donors were significantly higher than those of the AML patients (z=2.246; p=0.026). The median value; 65.70 (min=55.60; max=80.80) was determined for AML patients, while 89.05 (min=61.80; max=90.50) for healthy donors. For the other wells compared, there was no significant difference in the percentage of cell viability (p>0.05). The median value for AML patients was 73.50 (min=56.40; max=92.50), and the median value for healthy donors was 78.85 (min=76.60; max=85.40) (z=0.321; p=0.818). The percentage cellular viability values obtained from the wells in AML patients were not statistically significant (χ^2=2.184; p=0.823). The percent cellular viability values obtained from all wells were similar to those obtained from healthy subjects (χ^2=10.714; p=0.057) (Table 3).

Immunological Assays

Clonal division occurs in lymphocytes following mitogenic activation. The study examined the kinetics of lymphocyte stimulation with PHA and the impact of pediatric and normal bone marrow MSCs on activated lymphocytes using flow cytometry. We examined various lymphocyte subpopulations, particularly T cells phenotypically identified as $CD3^+CD69^+$, $CD3^+HLADR^+$, and $CD4^+CD25^+$, under various culture conditions.

MSCs of AML patients and healthy donors presented with the same morphological features, proliferation rates (z=1.074; p=0.394), and differentiation capacities. We analyzed T lymphocytes phenotypically defined as $CD3^+CD69^+$, $CD3^+HLADR^+$, and $CD4^+CD25^+$ in culture conditions by flow cytometry (Table 2 and Table 3). We observed differences between the different sets of co-cultures. We defined and compared different stages of lymphocyte activation in AML patients and healthy donors. The MNCs of healthy donors with PHA stimulation were found to be consistent with the

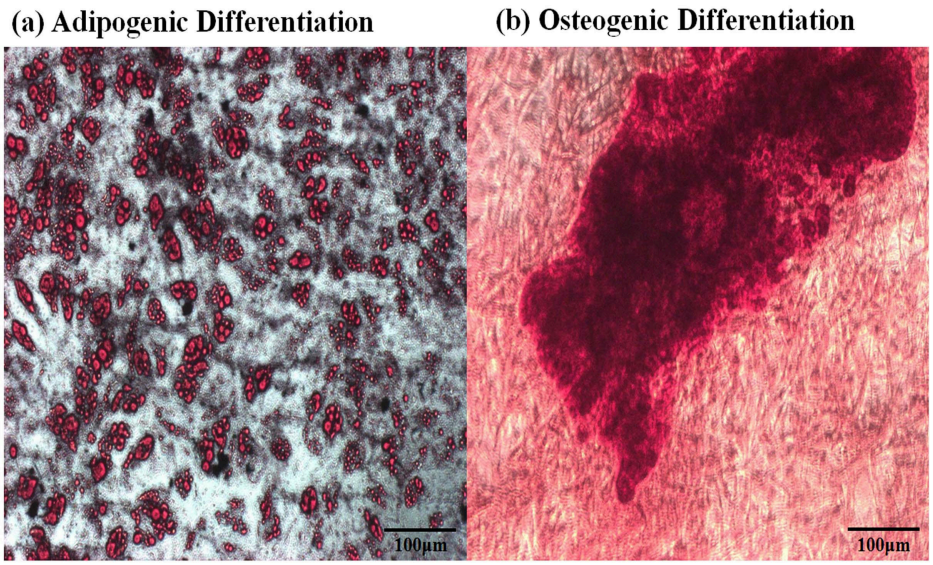


Figure 2. (a) Adipogenic differentiation of the MSCs of AML patients (stain: oil red o) (b) Osteogenic differentiation of the MSCs of AML patients (Olympus CKX41-40X)(Stain: Alizarin Red).

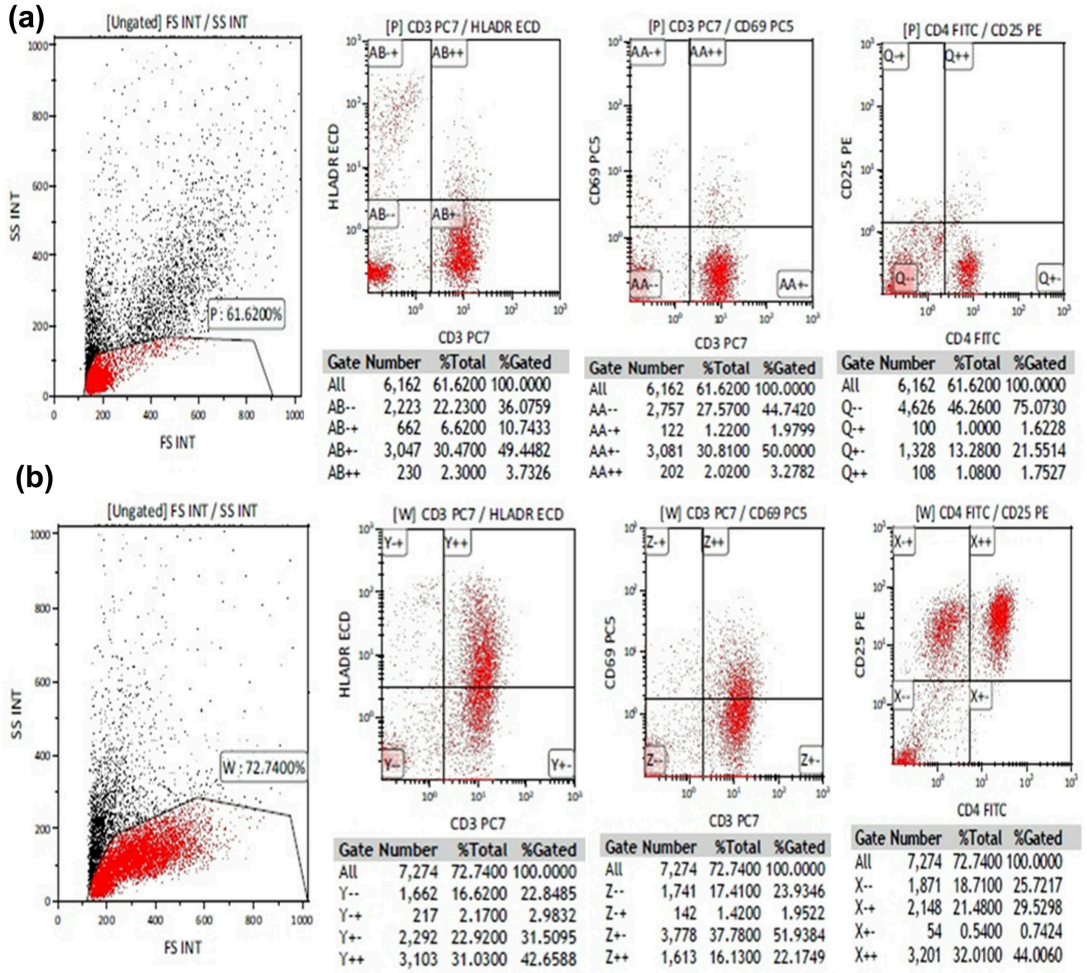


Figure 3. Flow-cytometric analysis of MNCs from induced with and without phytohaemagglutinin of AML group. Induced (a)Non-induced PHA-MNCs isolated from AML and, (b)induced by PHA-MNCs isolated from AML. The positive cells are indicated in the rectangular region.

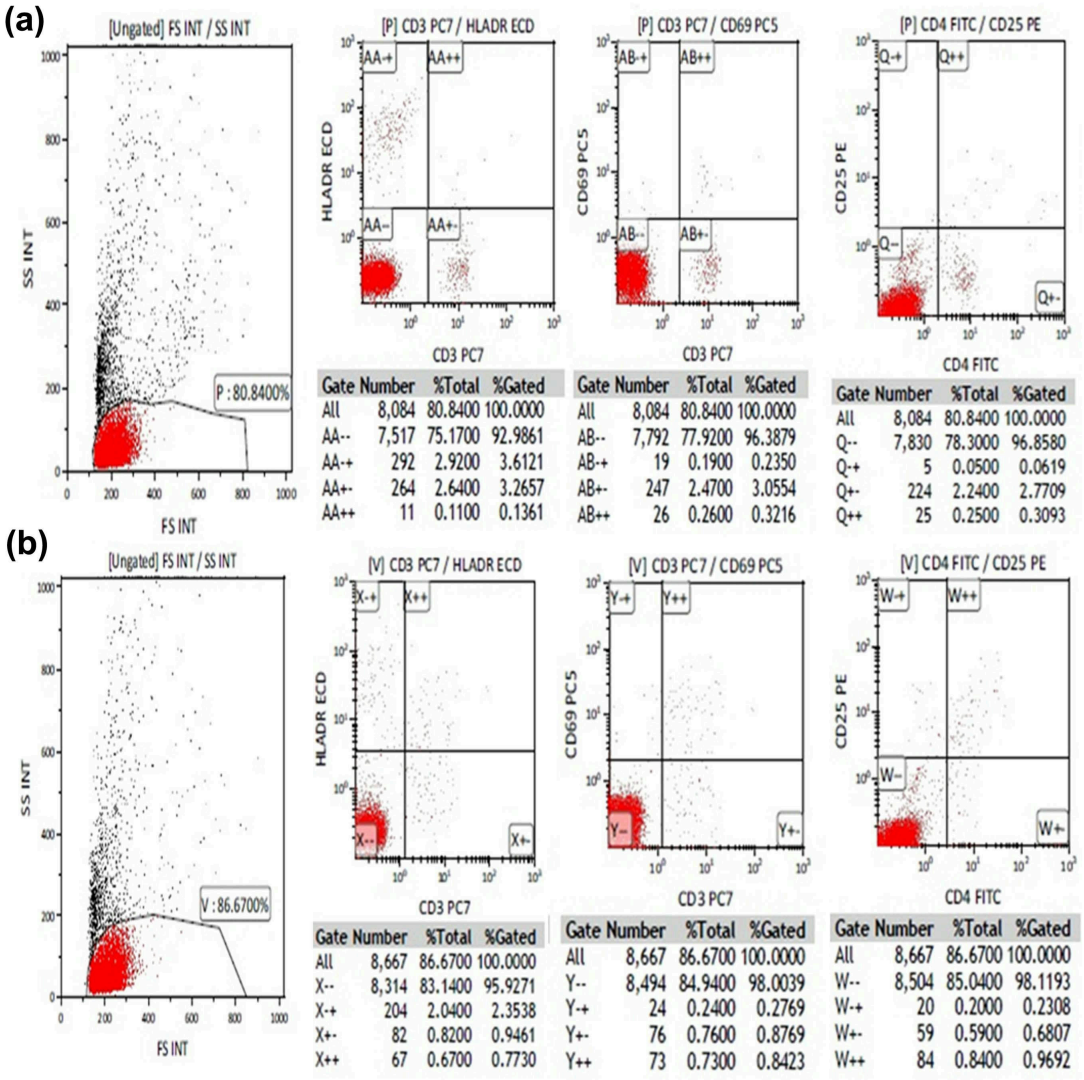


Figure 4. Flow-cytometric analysis of MNCs from induced with and without phytohaemagglutinin of pediatric control group. (a) Non-induced by PHA-MNCs isolated from control and, (b) Induced by PHA-MNCs isolated from control. The positive cells are indicated in the rectangular region.

literature regarding the expression of T-cell surface markers. However, the expression pattern of MNCs of AML patients indicated that they do not exhibit an effective immune response to PHA. The $CD3^+CD69^+$, $CD3^+HLA\text{-}DR^+$, and $CD4^+CD25^+$ MNCs of all groups of AML patients were similar before and after co-culture (Table 4).The mean percentage of $CD3^+CD69^+$ cells ($p=0.015$) were higher in D1 than in C2. After co-culture, the mean percentage of $CD3^+CD69^+$ cells was higher in F1 compared with D1 (Table 4) (Figure 3-5).

DISCUSSION

Pediatric leukemia, a major leukemia, has seen a 70% survival rate in recent years because of improved care, risk stratification, and intensive chemotherapy. MSCs are essential for tissue homeostasis and can differentiate into different cell types. They are found in many tissues like BM, adipose tissue, cord blood, and placenta (14-18).

This study characterized MSCs in children with AML, focusing on hematopoiesis in the BM microenvironment. Results showed morphological similarity between AML patients and healthy individuals, which is consistent with literature findings after standard cell culture applications. A previous study found no morphological difference in bone marrow-derived MSCs between patients with ALL and healthy individuals (12). The suitability of the variables in the study for normal distribution was assessed using the Shapiro-Wilk test. Because the variables did not show normal distribution and the number of subjects was small, median (minimum-maximum) values were used in the display of descriptive statistics. As additional information, mean ± standard deviation values were provided (Table 1).

Adipose tissue (AT)-derived cells, including BM and AT, exhibit diverse morphologies, including elongated, triangular, and discoidal flat shapes. After three passages, these cells

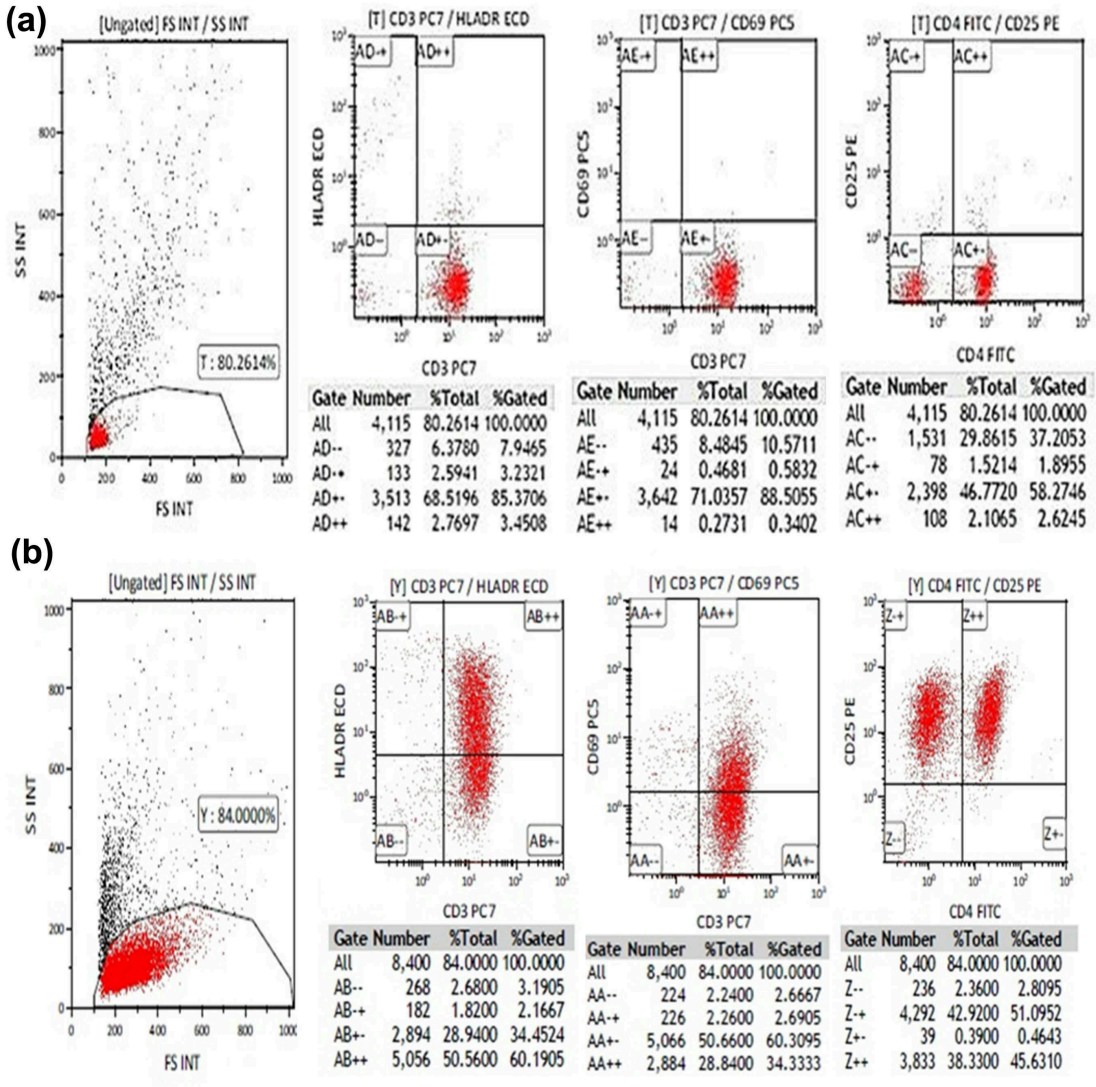


Figure 5. Flow-cytometric analysis of MNCs from induced with and without phytohaemagglutinin of healthy MNC group. (a) Non-induced PHA-MNCs isolated from healthy MNC donor and, (b) induced by PHA-MNCs isolated from healthy MNC donor. The positive cells are indicated in the rectangular region.

exhibit fibroblast-like morphology, abundant cytoplasm, and large nuclei, forming parallel models without morphological differences (19, 20). In 2013, Conforti et al. found that ALL (ALL-MSC=8.89 ± 0.87, p<0.001) had significantly lower proliferation capacity compared with healthy donors, as indicated by the Student's t-test, comparing PD values from passage 1 to passage 5 (HD-MSC=12.12 ± 1.09) (12). Compared with BM-derived MSCs, AT-derived MSCs showed higher proliferative capacity and higher numbers per passage in cumulative PD studies, with CPD values of 13.7 ± 0.5 and 17.2 ± 0.3, respectively (19). In our study, the Mann–Whitney U test was used to compare the values obtained from AML patients and donors in each well and to compare PD2 values for the cell proliferation assay (z=1.074; p=0.394) (Figure 1). According to cell viability analysis, when BM-MSC+Healthy PHA(+) MNCs were cocultured with healthy MSCs, it was found to be statistically significant (z=2.246; p=0.026) (Table 3).

PHA is a member of the lectin family and crosslinks with different cell membrane glycoproteins. PHA is also an inducing agent in polyclonal stimulation of lymphocytes (21). PHA mimics T lymphocyte signaling, but PKC activation persists despite crosslinking with TCR, CD2, and CD3. Calcium levels increase, but IL-2 from T cells is needed for antigen-presenting cell proliferation, and induced T lymphocytes require helper signaling from antigen-presenting cells (22-27). TNF-α transcription and gene expression are regulated by PHA, with CD69 being an early activation marker. After 4 h, transcription increases in T, B, and NK cells, resulting in increased affinity for IL-2 in the plasma membrane. ALL patients and healthy donors exhibited similar anti-inflammatory effects on MSCs, with HLA-DR expression beginning on the T cell surface after 40-60 h. *In vitro* inhibition of polyclonal-induced allogeneic peripheral blood mononuclear cells (PBMCs) in ALL-MSCs and healthy donor MSCs showed equal effects. Anti-inflammatory

Table 3. Comparison of cell viability between the healthy donor and AML groups

	Pediatric AML group		Healthy Group		z	p value
	Median (min-max)	Mean ± SD	Median (min-max)	Mean ± SD		
Control	70.95 (53.30-94.30)	70.98 ± 14.01	70.95 (56.30-79.00)	69.40 ± 8.80	0.161	0.937
BM-MSC+Healthy PHA(+) MNC	65.70 (55.60-80.80)	68.05 ± 10.04	89.05 (61.80-90.50)	84.60 ± 11.27	2.246	0.026
BM-MSC+AML PHA(+) MNC	73.50 (56.40- 92.50)	73.73 ± 13.16	78.85 (76.60-85.40)	76.40 ± 3.31	0.321	0.818
BM-MSC+Newly isolated MNC	73.10 (56.50-87.50)	72.20 ± 13.57	73.70 (51.30-84.40)	72.52 ± 12.49	0.320	0.818
BM-MSC+Healthy PHA(-) MNC	76.30 (64.70-81.80)	74.35 ± 7.23	73.60 (56.50-84.30)	72.45 ± 9.09	0.320	0.818
BM-MSC+AML PHA(+) MNC	78.55 (60.00-86.90)	76.38 ± 9.21	73.95 (63.60-86.90)	73.68 ± 8.51	0.643	0.589
χ^2; p value	2.184; 0.823		10.714; 0.057			

Abbreviations: Min:minimum; max:maximum; SD: Standard deviation; AML: Acute myeloid leukemia; BM: Bone marrow; MNC: Mononuclear cells; MSC:Mesenchymal stromal cells; PHA:Phytohemagglutinin; MNC: Mononuclear cells.

Table 4. Immunological assay determining activation markers of lymphocytes obtained from peripheral blood of AML and healthy groups

Activation markers	Control 1	Before co-culture		After co-culture			
AML Group	C1	B1	B2	E1	E2	E3	E4
$CD3^+CD69^+$	2.155 ± 1.619	5.46 ± 5.857	1.837 ± 2.186	3.77 ± 3.841	1.926 ± 2.238	17.228 ± 14.494	6.426 ± 9.47
$CD3^+HLA\text{-}DR^+$	19.455 ± 26.382	12.453 ± 18.669	5.44 ± 6.689	7.628 ± 10.304	3.302 ± 2.998	31.268 ± 19.212	4.434 ± 2.896
$CD4^+CD25^+$	4.525 ± 4.377	13.18 ± 14.776	10.023 ± 12.972	13.002 ± 7.987	9.35 ± 2.569	20.718 ± 12.179	6.382 ± 1.444
Healthy Group	Control 2	Before co-culture		After co-culture			
	C2	D1	D2	F1	F2	F3	F4
$CD3^+CD69^+$	0.42 ± 0.339	26.7 ± 6.985	16.233 ± 27.485	0	19.775 ± 19.127	17.895 ± 2.977	10.445 ± 8.04
$CD3^+HLA\text{-}DR^+$	2.455 ± 2.581	33.98 ± 23.516	9.353 ± 10.052	0	19.775 ± 19.127	34.525 ± 19.057	14.435 ± 6.951
$CD4^+CD25^+$	0.885 ± 0.587	26.26 ± 16.782	12.423 ± 16.954	32.5 ± 3.536	33.335 ± 47.143	22.745 ± 16.454	26.665 ± 9.426

AML Group (p values)	C1&B	C1&B2	B1&E1	B1&E2	B1&E3	B1&E4	B2&E1	B2&E2	B2&E3	B2&E4
$CD3^+CD69^+$	0.511	0.874	0.634	0.255	0.238	1.00*	0.463	0.958	0.127	0.571*
$CD3^+HLA\text{-}DR^+$	0.745	0.413	1.00*	0.3	0.225	0.358	0.571*	0.546	0.071	0.771
$CD4^+CD25^+$	0.498	0.619	0.983	0.572	0.461	0.321	0.696	0.91	0.284	0.535
Healthy Group (p values)	C2&D1	C2&D2	D1&F1	D1&F2	D1&F3	D1&F4	D2&F1	D2&F2	D2&F3	D2&F4
$CD3^+CD69^+$	0.015	0.496	0.014	0.585	0.204	0.094	0.486	0.887	0.941	0.8
$CD3^+HLA\text{-}DR^+$	0.171	0.432	0.148	0.533	0.98	0.355	0.3	0.468	0.138	0.585
$CD4^+CD25^+$	0.136	0.429	0.656	0.816	0.832	0.978	0.214	0.508	0.549	0.371

Abbreviations: C1 (control 1): AML MNC; B1: AML MNC PHA (+); B2: AML MNC PHA (-); E1: AML MSC+AML MNC PHA (+); E2: AML MSC+AML MNC PHA (-); E3:MSC+ Healthy MNC PHA (+); E4: AML MSC+ Healthy MNC PHA (-). C2 (control 2): Healthy MNC; D1: Healthy MNC PHA (+); D2: Healthy MNC PHA (-); F1: Healthy MSC+ AML MNC PHA (+); F2: Healthy MSC+ AML MNC PHA (-); F3: Healthy MSC+ Healthy MNC PHA (+); F4: Healthy MSC+ Healthy MNC PHA (-). **Note:** Data was presented mean ± standard deviation. Statistical significance was $p<0.05$. * Statistical analysis was performed using the Mann–Whitney U test.

cytokines and growth factors increased in both groups (12, 28). Studies have shown that MSC suppress T-lymphocyte proliferation when co-cultured with mixed lymphocytes. The suppression effects of MSCs are dose-dependent and independent of the MHC complex. Autologous and allogeneic MSCs suppress alloresponsiveness to T cells, but allogeneic

MSCs are not compliant to induce T cell proliferation when transfected with co-stimulatory molecules or IFN-γ. TNF, a cytokine produced by T cells, is highly receptive to allogeneic PBMCs, indicating their ability to respond to these cells (29).

The Mann–Whitney U test was used to examine whether $CD3^+CD69^+$, $CD3^+HLA\text{-}DR^+$, and $CD4^+CD25^+$ values obtained in PHA and non-PHA groups of AML patients differed. A comparison between the healthy MNC and healthy MNC PHA(+) groups revealed statistical significance (p=0.015). There was a statistically significant difference between the healthy MSC+AML MNC PHA(+) and healthy MNC PHA(+) groups (p=0.014). It has been shown that healthy MNCs activated with PHA create an inflammatory microenvironment for MSC, which transform their immunosuppressive properties into pro-inflammatory effects. These MSC-PHA co-cultures have cytotoxic effects against AML *in vitro*. This study aimed to elucidate the potential therapeutic implications of PHA-activated healthy mononuclear cells along with MSC for the treatment of acute myeloid leukemia. Healthy MNCs improve treatment responses against AML by enhancing anti-leukemia reactivity. hMNCs can be activated *ex vivo* using pharmacological agents that target the toll-like receptor (TLR) pathways and/or combine TLR agonists with proinflammatory cytokines. hMNCs activated by this approach elicit a more potent anti-leukemia immune response (30). PHA-induced healthy MNCs (hMNCs) are a promising approach for the treatment of AML patients. However, understanding the interplay between hMNCs and AML cells is crucial for their clinical application. The findings showed that PHA-induced healthy MNCs could effectively improve the transformation of unhealthy MNCs to healthy MNCs from AML patients, which was not observed in the PBMCs of healthy individuals (31).

The proliferative capacity of BM-MSCs from older individuals has been found to be faulty, with lower proliferation and survival compared with young healthy MSCs. Co-cultured with PHA-induced MNCs, these cells suppress B cell development and acquisition (32, 33). MSCs inhibit B cell proliferation, activation, differentiation, and Ig production, blocking B lymphocyte stimulation in the G0/G1 phase, linked to human BM-MSC and p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) 1/2 phosphorylation (18). The plastic surface of bone marrow from AML patients and donors shows fibroblast-like cells similar in morphology and adhesion to it. The study found no significant differences in PD2 values between the two cell groups. MSCs differentiated into adipocytes and osteocytes, demonstrating their potential for AML treatment. Flow cytometric analysis revealed no significant differences in $CD3^+CD69^+$, $CD3^+HLA\text{-}DR^+$, and $CD4^+CD25^+$ T-cell activation markers between MNC cell cultures from AML patients and healthy donors. AML donor and AML patients' MSCs were obtained, revealing significant differences in interaction between the two groups. No significant difference was found between AML patients or donors. Co-culture of MSCs from AML patients and healthy individuals is expected. The functionalities of MSCs vary with donor age, making it crucial for physicians to define detailed immunological markers considering donor age to understand the effects of MNCs on MSCs in the bone marrow microenvironment.

CONCLUSION

Oncogenic mutations are responsible for the development of AML, a stem cell disease. Despite normal functionality, cell-cell interaction in bone marrow tissue influences disease prognosis, and further immunological analyses are required for a better understanding.

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