ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Response Of Myeloid Dendritic Cells In Tumor Microenvironment Of Breast Cancer: Alteration In Secretory Function.

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ABSTRACT

Dendritic cells (DCs) are bone-marrow derived cells circulated to capture various types of antigens. DCs divided into two subtypes; myeloid dendritic cells (mDCs) and pDCs. Cytokines and chemokines produced by DCs are essential to define their role in innate and adaptive immune responses. The aim of present study was to isolate and characterize tumor associated myeloid dendritic cells (TAmDCs) in breast cancer patients. Blood from peripheral and axillary vein tributaries blood was collected during breast cancer surgery and mDCs were separated from total leukocytes by immunemagnetic beads technique and were cultured overnight. Media conditioned by mDCs were collected and subjected to cytokine profiling using cytokine antibody array . We found reduction in Cytokine profiling of TAmDCs isolated from breast cancer patients compared to normal mDCs isolated from healthy volunteers and we found that secretory products (cytokines /chemokines/growth factors) derived from tumor microenvironment mDCs isolated from axillary tributaries was lower than peripheral mDCs isolated from peripheral blood of breast cancer patients. In conclusion, the present results suggest that tumor microenvironment derived factors evade immune response by impairment secretory function of mDCs .Targeting the major tumor microenvironment derived factors may be used to inhibit suppression in DCs secretory function.

Keywords: Myeloid dendritic cells, Cytokine array, Breast cancer, Tumor microenvironment.

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ISSN: 0975-8585

INTRODUCTION

Dendritic cells (DCs) are bone-marrow derived cells characterized by unique dendrites and by expressing high levels of major histocompatibility complex (MHC) class II products [1-3]. DCs distributed in the circulation, lymphoid and non-lymphoid organs to capture various types of antigens [3, 4]. DCs are characterized by presenting antigens to naïve T cells, as they encounter antigens in peripheral tissues and migrate to lymphoid organs to present the captured antigens to naïve T cells. This makes them vary from other antigen presenting cells (APCs) and induce the T cells to initiate an effective immune response [1, 5]. Under steady state conditions, DCs divided into two important subtypes; plasmocytoid dendritic cells (pDCs) and myeloid dendritic cells (mDCs). These two subtypes originate from two progenitors; lymphoid progenitors can differentiate to all thymic and splenic DCs subsets. Moreover, myeloid progenitors can give rise to macrophage-DC progenitors in the bone marrow that will further give rise to monocytes and common DCs progenitors [6]. The common DCs progenitors differentiate into both subsets (pDCs and cDCs), monocytes can differentiate to DCs during inflammation and DCs derived from these monocytes known as inflammatory DCs [7]. DC subsets are characterized by high expression of the HLA-DR molecule, absence of lineage marker expression (CD3, CD14, CD19, CD56), and specific expression of CD11c on mDCs [8-10]. DCs induce efficient anti-tumor response through several steps (i) recognition of various tumor molecules by DCs (ii) DCs activate and induce recruitment of other leukocytes such as macrophages, natural killer (NK) and NKT cells (iii) DCs migrate to lymph nodes to present captured tumor antigens to T lymphocytes, afterwards T cells activate, expand and migrate to tumor sites to destroy the transformed tumor cells [11, 12]. Large panel of cytokines and chemokines secreted by mDCs subsets in response to several damage-associated molecular patterns (DAMPs) [13-15]. The major cytokines secreted by mDCs were TNF-α/CXCL8 (IL-8)/IL-6/ CCL3/CCL4 and, to a lesser extent, IL-1β; these have a primary role in the induction of inflammation [16-18]. However, spontaneous obstruction in elimination steps leads to impairment in malignant destruction process [19, 20]. Several studies have evinced sharp phenotypic and functional impairment of DCs in breast cancer patients [21, 22]. DCs isolated from blood of breast cancer patients express low levels of co-stimulatory molecules, therefore their ability to stimulate T- cells has been reduced and their secretion of some cytokines such as IL-12 has also decreased [22]. IL-12p70 is one of the hall-mark cytokines involved in initiation of the adaptive Th1 immune responses. Cytokines and chemokines produced by DCs are essential to define their role of DCs in innate immune responses and Moreover, the subsequent adaptive immune [23-26]. Therefore, DCs play critical role in regulating the final outcome of the immune response [27, 28]. Levels of cytokines Secreted from mDCs differ according to type of stimulation. Therefore, to investigate the changes in the cytokine repertoire of mDCs isolated from breast tumor microenvironment, we compare the secretory products (condition media (CM) derived factors) of tumor associated myeloid dendritic cells isolated from breast cancer patients' axillary tributaries blood (from tumor microenvironment) and peripheral blood versus secretory products (CM derived factors) of healthy volunteers.

MATERIAL AND METHODS

Patients

For the purpose of patient's enrollment in this study, Institutional Review Board (IRB) approval from the ethics committee of Ain-Shams University, Cairo, Egypt was obtained. Patients were enrolled from outpatient breast clinic of Ain-Shams University hospitals. Before participation, all patients signed consent form including approval for publication of the study. We enrolled 30 women diagnosed with breast cancer , the patients should be pre-operatively clinically diagnosed as breast cancer cases by clinical examination, mammography and ultrasound [29] Only non-inflammatory (non-IBC) breast cancer cases that did not receive pre-operative neoadjuvant chemotherapy were enrolled in this study. Patients who had any viral infection, autoimmune diseases, pregnant women or lactating women and those who received pre-operative chemotherapy were excluded from this study.

Blood sample collection and isolation of tumor associated myeloid dendritic cells (TAmDCs)

During modified radical mastectomy, 10-15 ml blood that had drained from the tumor microenvironment through axillary tributaries and peripheral blood was collected by the surgeon in heparinized tubes as previously described by [30] . After each surgery blood was transferred directly to the laboratory to isolate myeloid dendritic cells (mDCs) from the whole blood. Peripheral blood from healthy

volunteers were also collected in heparinized tubes and transferred to laboratory to isolate normal mDCs. Equal amounts of collected blood were diluted with equal amounts of PBS (Cat.no.BE17-516F) at room temperature (RT), PH 7.2. Mononuclear cells (PBMC) were separated according to density, Ficoll-Hypaque density gradient centrifugation was used and ~1:2 diluted bloods layered on the surface of lymphocyte separation media (LSM) (Cat.no. 17-829E) and immediately centrifuged at 1500 rpm for 30 mins. This allows the erythrocytes and the granulocytes to sediment through the medium while the mononuclear cells are concentrated as band called buffy coat at the sample/medium interface where the cell density matches that of the surrounding solution. Buffy coat layer was aspirated and washed twice in PBS. Red blood cells (RBCs) lysis buffer (Cat.no.10-548E) was added to mononuclear cells at necessity. We purified mDCs from mononuclear cells using"Human Myeloid dendritic cells Negative selection Enrichment Kit" (StemCell Technologies, Vancouver, Canada). Steps of mDCs isolation were followed as described in the kit guidelines and began with $2x10^6$ of viable mononuclear cells suspended in 1 ml recommended media (PBS + 2 V/V % Fetal bovine serum (Cat.no .A 15-151) + 1mM EDTA) in polystyrene tubes according to the kit (Cat. no.19061) and incubated with 15 µl/ml of anti-Human CD32 (Fcγ RII) blocker and 50µl /ml of human myeloid DC Enrichment cocktail antibody for 30 mins at RT followed by incubation for 10 mins with 100 µl/ml EasySep™ D Magnetic Particles. The cell suspension was diluted with recommended media and placed into EasySep™ magnet for 5 mins. The magnetically labeled unwanted cells will remain bound inside the original tube, held by the magnetic field of the EasySep™ Magnet. Purity of isolated cells was confirmed by flow cytometric analysis [31] and found to contain 90–92% $CD11c^+$ mDCs. The negatively selected, enriched cells were seeded overnight in RPMI-1640 media with 5 mM L-Glutamine (Sigma) + 100 IU/µg/ml Pencillin/Streptomycin (Sigma). Media conditioned by mDCs secretions were collected, aliquoted and stored at −80 ^oC for cytokine profiling and further studies.

Determination of protein concentration in mDCs condition media

Total protein concentration was determined by Bradford assay [32] which relies on the formation of complexes between Coomasie Brilliant Blue G-250 dye and proteins in solution. This binding leads to shift in absorption of dye from 465 nm to 595 nm. Bovine serum albumin (BSA) (Cat.no. A2153-100G) is used to construct a standard curve. Serial standard dilution (20 µl) (working range 100-2000 µg/ml), as well as samples (mDCs condition media) were added into test tubes and 1 ml Bradford (1X) reagent (Cat. no.P150126) was added in each tube, vortexed and incubated for 5 mins at RT in the dark. The standard curve was constructed by plotting the absorbance at 595 nm versus protein concentration of each protein standard. By using standard curve, the protein concentration of unknown samples was determined.

Cytokine profiling of mDCs conditioned media by cytokine array

Media conditioned by mDCs were subjected to profiling using RayBio™ human cytokine antibody array 3 that simultaneously detects 42 cytokines per sample. Culture media without mDCs secretions were run in parallel as negative control. Experimental steps were conducted according to the manufacturer's instructions as previously mentioned [33]. Membranes were incubated with 1 ml of sample (known protein concentration (2000µg/ml) of mDCs secretory products) at 4^oC with gentle shaking overnight. Samples were washed 3 times (5 mins each) with 2 ml of 1X wash Buffer I and II at RT with shaking followed by overnight incubation with 1 ml of diluted biotin-conjugated antibodies at 4^oC and with gentle shaking. Washing was done once more and 1.5 ml of 1X HRP-conjugated streptavidin was added to each membrane and was kept for 2 hrs at RT then washed. Detection was done by using chemiluminescence detection buffer C and D provided with the kit against X-ray CL-X Posure film (Thermo Scientific, USA) for 30 seconds and the X-ray processing was done using Kodak developer and Kodak fixer (Kodak, France). Signal intensity values representing detected cytokines were subtracted from the back-ground and normalized to positive controls on the same membrane using ImageJ software (National Institutes of Health, MD, USA) as we described previously [33]. Signal intensity values of each cytokine assessed in conditioned media of mDCs isolated from peripheral and tumor microenvironment blood (blood from axillary tributaries) of breast cancer patients and in conditioned media of normal mDCs isolated from healthy volunteers are presented as mean± standard deviation (SD). Significant differences in levels of secretion of cytokines/chemokines/growth factors between breast cancer patients versus healthy volunteers were assessed using Student's t-test.

ISSN: 0975-8585

RESULTS

Cytokines array profiling was done for the media used in mDCs preparation, for both healthy volunteers and patients. Figure (2) shows cytokine profile in the media used in mDCs preparation to insure that there is no cytokines in preparation media.

Normal mDCs isolated from healthy volunteers secrete large panel of cytokines, chemokines and growth factors with elevated levels. TAmDCs from breast cancer patients secret lower levels of cytokines and chemokines compared to healthy volunteers figure (3) .Tumor microenvironment mDCs isolated from venous drainage of axillary tributaries from breast cancer patients secret lower levels of cytokines than peripheral mDCs isolated from peripheral blood of breast cancer patients . From normal mDCs , EGF (Epidermal growth factor) was the highest secreted followed by IL-8 (interleukin-8) (CXCL8) as the second most secreted , MCP-1(monocyte-chemoattractant protein-1) , IL-1α (interleukin-1 alpha), IL-1β (interleukin-1 beta) , oncostatin M, leptin, MCP-2 (monocyte chemotactic protein 2), MCSF(macrophage colony stimulating factor) , IFN- γ (interferon-gamma) . IL-7(interleukin-7) was the lowest secreted cytokine.

Peripheral mDCs secrete lower levels of cytokines and the panel of highest secreted cytokines was different from normal mDCs and regulated from the highest as follows; RANTES (regulated on activation normal T cells expressed and secreted) followed by IL-8, MCSF, EGF, GRO (growth regulated oncogene), IFN-γ, ENA-78 (epithelial neutrophil-activating protein 78), PDGF BB (platelet –derived growth factor), IL-1α, oncostatin M and the lowest secreted was also IL-7.

Table 1: Signal intensity (SI) of cytokines secreted from myeloid dendritic cells (mDCs) isolated from healthy volunteers and breast cancer patients

	Signal intensity (SI) (Mean ± SD)		
Cytokines	Normal	Peripheral	Axillary tributaries
IL-1α	131201 ± 7815.651	46677± 685.8936	24455 ±1968.585
IL-1β	124098 ± 911.460	43771± 1667.358	27540.5 ± 631.446
Oncostatin M	123146.5 ± 6576.093	46674.5±1392.293	32250 ± 393.151
MCSF	120690 ± 5111.675	54912 ± 367.695	38071.5 ± 177.484
IFN- γ	120414 ± 1651.094	48255 ± 2609.224	28347 ± 110.3087
TNF-α	118051.5 ± 5528.161	38906 ± 1244.508	35005.5 ± 358.5031
TNF-β	117021.5 ± 3286.632	41754 ± 1811.608	25542.5 ± 925.6028
TGF-β1	113323.5 ±977.222	43433 ± 130.108	29383 ± 1385.929
IL-3	113282 ± 13103.4	46170 ± 1134.199	38528 ± 370.524
IL-15	113198.5 ±3682.612	39417.5±1477.146	25304.5 ± 79.903
GM-CSF	110985 ± 1014.698	41630.5±1474.318	19643.5 ±55.8614
IL-6	108374 ± 171.827	32729 ± 2426.79	19175.5 ±1358.352
SCF	102899 ± 21406.24	36070 ± 1670.186	29247 ± 571.342
IL-2	97045 ± 116.673	30666 ± 608.112	19983 ± 1207.738
IL-13	96496.5 ± 595.384	40158.5±1757.164	14627 ± 700.036
IL-10	94427 ± 1533.715	39384.5 ± 3160.06	29845.5 ±712.056
IL-5	91665 ± 9054.502	35463.5±382.545	13816 ± 540.229
IL-4	88529 ± 6432.55	36779.5 ± 1982.02	20881 ± 654.781
IL-12p40p70	81279 ± 720.5418	36962.5±2725.897	22208 ± 535.987
GCSF	77449 ± 1132.078	28825 ± 1182.283	15738.5 ± 433.456
IL-7	69504.5 ± 7334.112	24553 ± 3361.586	16144.5 ±2047.074

Signal intensity (SI) of cytokines secreted from tumor associated mDCs (TAmDCs) isolated from axillary tributaries blood and peripheral blood of breast cancer patients, signal intensity of normal mDCs cytokines isolated from healthy volunteers. Data represented as mean ± SD (standard deviation) . IIL-1α (interleukin-1α), IL-1β (interleukin-1β), MCSF (macropage colony-stimulating factor), IFN-γ (interferon–gamma), TNF-α (tumor necrosis factor alpha), TNF-β (tumor necrosis factor alpha), TGF-β1 (tumor growth factor beta1) , IL-2, IL-3 , IL-4 , IL-5 , IL-6 , IL-7, IL-10, IL-12p40p70 , IL-13 , IL-15 (interleukin 2,3,4,5,6,7,10,12,13,15), GM-CSF (granulocyte-macrohage colony-stimulating factor) , GCSF (granulocyte-colony stimulating factor).

ISSN: 0975-8585

Table 2: Comparison between signal intensity (SI) of chemokines secreted from normal mDCs and breast cancer patient's mDCs (from axillary tributaries and peripheral blood.

	(Signal intensity) Mean ± SD		
Chemokines	Normal	Peripheral	Axillary tributaries
IL-8 (CXCL8)	137670.5 ± 31989.51	55825.5 ± 4560.132	42808.5 ± 1467.247
MCP-1 (CCL2)	132701.5 ± 10859.75	34063.5 ± 1870.297	19009 ± 1012.577
MCP-2 (CCL8)	121295.5 ± 21435.23	43880.5 ± 1017.527	24170 ± 3405.426
ENA-78 (CXCL5)	117774 ± 3285.925	47122.5 ± 1429.063	27534.5 ± 570.6352
GRO	111897.5 ± 698.6215	49345.5 ± 835.0931	31403.5 ± 1027.426
SDF-1 (CXCL12)	110417 ± 10604.48	45368 ± 1619.275	7949 ± 5.656854
RANTES (CCL5)	107848 ± 19296.24	75849.5 ± 1747.261	52067 ± 461.0336
MDC	107811 ± 8855.098	42548 ± 1202.082	25668 ± 173.9483
TARC	106362 ± 5456.743	42694 ± 752.361	27153 ± 316.7838
GRO-α (CXCL1)	103339 ± 11489.78	37333 ± 1756.453	26413 ± 1951.615
MIP-1 δ	93292.5 ± 10308.2	37088 ± 3586.446	23011.5 ± 1270.671
MIG	86204.5 ± 168.2914	32966.5 ± 1272.085	24743 ± 1969.999
MCP-3	86173 ± 3786.557	26907 ± 337.997	20259.5 ± 1948.079

Data represented as mean ± SD

IL-8 (interleukin-8), MCP-1 (monocyte chemotactic protein-1), MCP-2(monocyte chemoattaractant protein 2), ENA-78 (epithelial neutrophil-activating protein 78), GRO (growth regulated oncogene), SDF-1(stromal derived factor-1), RANTES (regulated on activation normal T cells expressed and secreted), MDC (macrophage derived chemokine), TARC (thymus and activation regulated chemokine), GRO-α (growth regulated oncogene alpha) , MIP-1 δ (macrophage inflammatory protein - 1) , MIG (Monokine induced by gamma interferon) , MCP-3 (monocyte chemotactaic protein 3).

Table 3: Signal intensity (SI) of growth factors, hormones and enzymes derived from condition media (CM) of mDCs isolated from healthy volunteers and breast cancer patients (peripheral blood and venous drainage of axillary tributaries).

	Signal intensity (SI) (Mean ± SD)		
Growth factors, Hormones Enzymes	Normal	Peripheral	Axillary tributaries
EGF	145284 ± 13657.77	53470 ± 444.0631	40894 ± 1210.567
Leptin	121367.5 ± 3478.965	43472.5 ± 3826.155	27588.5 ± 456.084
IGF-1	112560.5 ± 6902.776	40460.5 ± 85.559	29921 ± 3459.166
Angiogonin	110065 ± 977.9287	42217.5 ± 499.924	25463 ± 3046.216
Thrombopoietin	109122.5 ± 506.2885	36037.5 ± 2116.371	26134.5 ± 1173.09
VEGF	95814 ± 311.8341	28938 ± 3252.691	24208.5 ± 751.654

Data represented as Mean ± SD

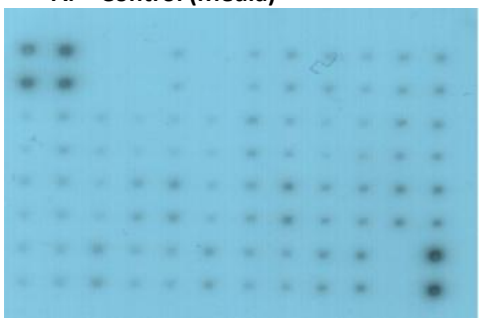
EGF (Epidermal growth factor), IGF-1(Insulin-like growth factor) , VEGF (vascular endothelial growth factor).

	a	b	c	d	e	f	g	h	i	j	k	l
1	Pos	Pos	Neg	Neg	ENA-78	GCSF	GM-CSF	GRO	GRO-α	I-309	IL-1α	IL-1 β
2	Pos	Pos	Neg	Neg	ENA-78	GCSF	GM-CSF	GRO	GRO-α	I-309	IL-1α	IL-1 β
3	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12 p40p70	IL-13	IL-15	IFN-γ
4	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12 p40p70	IL-13	IL-15	IFN-γ
5	MCP-1	MCP-2	MCP-3	MCSF	MDC	MIG	MIP-1 δ	RANTES	SCF	SDF-1	TARC	TGF- β1
6	MCP-1	MCP-2	MCP-3	MCSF	MDC	MIG	MIP-1 δ	RANTES	SCF	SDF-1	TARC	TGF- β1
7	TNF-α	TNF-β	EGF	IGF-I	Angiogenin	Oncostatin M	Thrombopoietin	VEGF	PDGF BB	Leptin	Neg	Pos
8	TNF-α	TNF-β	EGF	IGF-I	Angiogenin	Oncostatin M	Thrombopoietin	VEGF	PDGF BB	Leptin	Neg	Pos

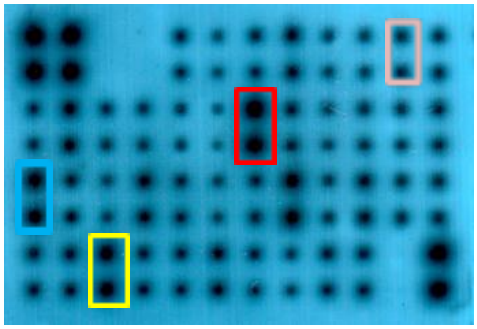
Figure (1): Map of Raybio™ human Cytokine antibody array 3

ISSN: 0975-8585

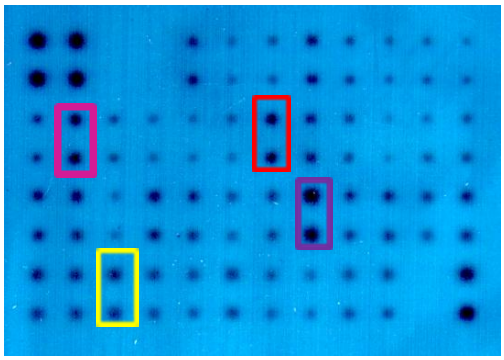
A. Control (Media)



B. Normal (Healthy volunteers)



C. Axillary tributaries (patients)



D. Peripheral (patients)

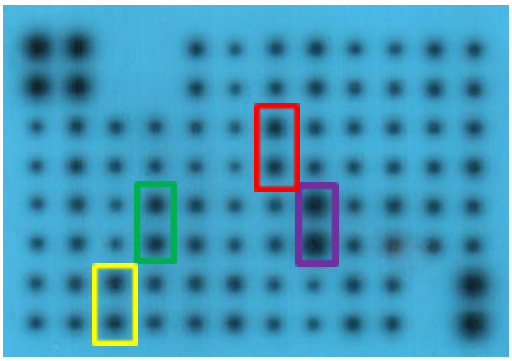


Figure (2): Profiling of mDCs derived factors (secretory products) using RayBio™ Human Cytokine Antibody Array 3 . (A) Representative array of control media in which the normal mDCs and TAmDCs were prepared and no cytokines were detected. (B) Representative array of (normal mDCs) secretory products from healthy volunteers. (C) and (D) are representative arrays for the cytokines profile of TAmDCs secretory products from breast cancer patients.(C) Representative array of (tumor-microenvironment mDCs secretory products : TAmDCs isolated from axillary tributaries of breast cancer patients) while (D) Representative cytokines array of (peripheral mDCs : TAmDCs isolated from peripheral blood of breast cancer patients) .

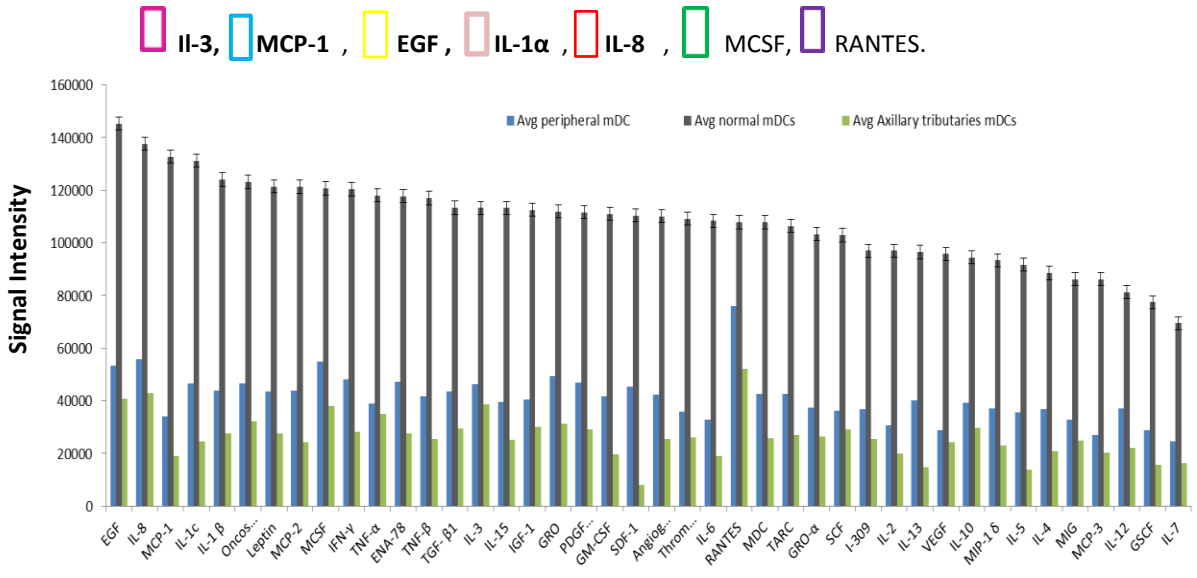


Figure (3): Comparison between cytokine profiles of mDCs isolated from healthy volunteers versus breast cancer patients. Bars represents signal intensity value of each cytokine secreted by mDCs isolated from breast cancer patients mDCs (peripheral mDCs and tumor-microenvironment mDCs) and normal mDCs isolated from healthy volunteers as detected by RayBio TM human cytokine antibody array 3 and intensity value quantified by ImageJ software .

Tumor microenvironment mDCs isolated from axillary tributaries secret the lowest levels of cytokines, RANTES, IL-8, EGF were also the highest secreted as peripheral mDCs but with inferior levels followed by IL-3 (interleukin-3), MCSF, TNF-α (tumor necrosis factor alpha), oncostatin M, GRO, IGF-1 (insulin-like growth factor), IL-10 (interleukin-10). SDF-1(stromal derived factor-1) was the lowest secreted chemokine by tumor microenvironment mDCs.

Only two array of cytokines profile of TAmDCs derived factors (secretory products) from breast cancer patients are included in figure (2) which represents the major profiling pattern among all patients' samples. Figure (1) shows the map of Raybio™ human cytokines antibody array 3 used in this study. To compare between secretion levels of different cytokines among patients samples arrays and healthy volunteers arrays, the density value of each cytokine in each array was calculated by using Image j software analysis tool. The histogram in figure (3) is based on these data and table (1) which represent density values of cytokines, table (2) represent density values of growth factors and table (3) represent density values of chemokines represented as (mean ± SD).

DISCUSSION

We have studied the pattern of cytokines expressed by TAmDCs populations isolated from breast cancer patients either from venous drainage of axillary tributaries or from peripheral blood. The results demonstrate that ; 1) normal mDCs isolated from healthy volunteers secrete large panel of cytokines, chemokines and growth factors with elevated levels 2) TAmDCs from breast cancer patients secret lower levels of cytokines and chemokines compared to healthy volunteers 3) tumor microenvironment mDCs isolated from venous drainage of axillary tributaries from breast cancer patients secret lower levels of cytokines than peripheral mDCs isolated from peripheral blood of breast cancer patients . EGF (Epidermal growth factor) was the highest secreted from normal mDCs followed by IL-8 /CXCL8 while RANTES was the highest secreted chemokine by both peripheral and tumor microenvironment mDCs followed by IL-8 but with lower lever secreted from tumor microenvironment mDCs than peripheral mDCs. Our results are in agreement with a previous studies reporting diverse panel of growth factors, cytokines and chemokines that are immunologically relevant secreted by DCs [13, 14, 15, 34]. Initial stimulation of immature DCs induces them to produce wide range of inflammatory chemokines including CCL2 (MCP-1) , CCL3(MIP-1α), CCL4 (MIP-1β), CCL5 (RANTE), CCL7 ,CCL8 , CXCL8 (IL-8) and CXCL10 (IP-10) [35-38], however , they lose their capacity to produce these chemokines after maturation and start to produce chemokines attracting B and T lymphocytes such as CCL17 (TARC), CCL19 (MIP-3β), CCL20,CCL21 and CCL22 (MDC) [39-41]. Moreover, pattern of cytokine expression of blood-derived DCs was analyzed by semiquantitative RT-PCR and large array of cytokine mRNAs produced including IL-1β , IL-6, IL-13 , IL-15, tumor necrosis factor alpha (TNF-α), tumor growth factor beta (TGF-β) that contribute to T cells priming or contributing to T cell maturation (IL-7, IL-18, IL-12) [17] [34,42].Rate of survival of DCs increased when family of proteins termed TNF expressed on activated T cells ligated with TRANCE/ RANK and CD40 receptor designated on DCs [43-45] . Ligation of CD40 up-regulate secretion of several cytokines and chemokines such as IL-8, IL-12 and macrophage inflammatory protein (MIP-1α), (MIP-β) [46,47].Uptake of particle-adsorbed Ag by DCs increases transcription of IL-1α and both subunits of IL-12(p35 and p40) [44,48,49,50,51]. IL-12 is heterodimeric cytokine produced mostly by activated mDCs which plays essential role in the differentiation and expansion of Th1 cells [48, 51]. IL-23 was found to share some features with IL-12 as they have common p40 subunit [42]. Levels of cytokines Secreted from mDCs differ according to type of stimulation i.e. monocyte-derived DCs from multiple sclerosis (MS) patients, secrete equivalent amounts of IL-12 but more IL-23 compared to healthy controls [42, 52, 53]. IL-7 was found supporting B cells growth while IL-18 synergizes with IL-12 to induce production of IFN-g by T h cells [34, 54, 55] and to block IgE production from B cells [34, 56]. PMA-ionomycin activation up-regulates secretion of IL-13 and GM-CSF [34]. IL-13 could indirectly favor the commitment of naive T cells toward Th2 pathway; DC-derived IL-13 is likely to contribute to the regulation of B cell proliferation [57, 58]. Monocyte derived DC as well as blood DC [59] express IL-10, while Langerhans cells fail to express IL-10 mRNA [60] which support the concept that different populations of DCs have independent pathways to develop [61,62]. Bone marrow derived immature DCs expressed greater levels of TNF-α, IL-1α, IL-1β, TGF-β1 and macrophage migration inhibitory factor (MIF) transcripts/protein [55]. However, limited number of chemokines, such as macrophage-derived chemokine/CCL22, TARC/CCL17, and PARC/DC-CK1/macrophage inflammatory protein-4/CCL18, are secreted by both immature blood myeloid DCs and monocyte-derived DCs[35,39,63,64].Up-regulation of CCL22 and CCL17 in maturing DC has been investigated in several studies and CCL18, together with CCL22, CCL17, and CXCL8, represents one of the most abundantly secreted chemokines by DC [35,39,65]. After spontaneous differentiation in culture, DCs increased the levels of

IL-6 and IL-15 mRNA and transcribed mRNA for IL-12p35, IL-12p40, and IL-18 de novo and similar findings were detected at the protein level [63]. Alternation in cytokine repertoire observed after DCs differentiation with CD40 cross-linking or lipopolysaccharide (LPS) [63]. Both of them up-regulated levels of TNF-α , IL-6, IL-12p70 and IL-15 transcripts/intracellular protein but only LPS was distinctly increased expression of IL-1α,IL-1β and IL-12p35 therefore , only LPS was able to shift differentiation of naïve T helper cells to Th1 cells [63]. IL-1α, IL-6 and oncostatin M (OSM) are members of IL-6 family, they exhibit pro- as well as anti-inflammatory effects and play essential role regulating innate and adaptive immune responses [42]. IL-6 with TGF-β induces differentiation and proliferation of Th17 and also induces differentiation of B lymphocytes into antibody-producing plasma cells [66, 67]. DCs have several classes of surface and intracellular receptors which enable them to encounter pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) [68]. Nod-like receptors (NLRs) are one of recognition receptors that reduce secretion of some inflammatory cytokines such as (IL-1β and IL-18), once activate with uric acid as one of the DAMPs [42, 69]. CCL2 is important chemokine regulating the recruitment and migration cells of the monocyte-macrophage system. Monocyte chemoattractant protein-1 (CCL2/MCP-1) modulates T cell immune response and causing their switch from Th0 to Th2 with predominant secretion of IL-4 [70-72]. RANTES/CCL5 is a chemokine that regulates process of wound healing and mediates chemotactic activity for some leukocytes including lymphocytes and monocytes; CCL5 induces angiogenesis and leukocytes migration [72, 73]. VEGF (vascular endothelial growth factor) is essential mediator in T cells priming and polarization to Th1 and Th17 and also up-regulates their pro-inflammatory cytokines secretion [74]. The main function of (VEGF) is promoting endothelial cell proliferation, differentiation and migration, VEGF also mediating recruitment of inflammatory cells and induces expression of co-stimulatory molecules on these recruited mononuclear cells [72,74,75].

CONCLUSION

DCs have been attracting a lot of scientific and clinical attention for their effective role in tumor immunity and for their potential to be used as adjuvants in tumor vaccination**.** present study define the role played by tumor microenvironment to impair secretory function of DCs. Therefore, the need for generation of *ex vivo*-educated DCs arose due to dysfunction of endogenous DCs in cancer patients avoiding for any possible interventions in therapeutic efficacy**.** Targeting the major tumor microenvironment derived factors may be used to inhibit suppression in DCs secretory function.

ACKNOWLEDGEMENTS

This work was conducted at Cancer Biology Research Laboratory(CBRL), Department of Zoology, Faculty of Science, Cairo University, Egypt.

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