Studying modulatory effects of Prostaglandin E2 on dendritic cells-induced apoptosis by breast cancer cells

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Abstract: Dendritic cells (DCs) are antigen-presenting cells that orchestrate the innate and adaptive immune response and play an important role in immune response against tumor cells. DCs were found dysfunctional and apoptotic in cancer microenvironment. Prostaglandin E2 (PGE2) is a bioactive lipid driven by cyclooxygenase (Cox) Cox-1 and Cox-2 that elicits a wide range of biological effects associated with inflammation and cancer. The aim of this paper is to investigate the role of PGE2 and tumor soluble factors in controlling DCs phenotype expression, cytokine secretion and apoptosis. Monocytes-derived DCs were generated *in vitro,* then they were challenged with PGE2 and breast cancer supernatant (CSN) from MCF-7 cell line culture. DCs morphology was studied using bright field microscopy, phenotype characteristics and apoptosis using flow cytometry, and ELISA techniques were used to determine interleukin-10 (IL-10) and interleukin (IL-12p70) production. **Results**: CSN-treated DCs showed significantly low expression of maturation marker CD83, costimulation marker CD86 and high expression of CD80 in comparison to unstimulated DCs. By contrast, PGE2-stimulated DCs showed downregulation of CD80 and upregulation of CD86, whereas CD83 remained unchanged compared with unstimulated DCs. On the other hand, CSN-P (mixture of PGE2 and CSN) stimulated DCs showed significant expression of CD83, CD80 and CD86, however the expression of CD80 was decreased compared to that of CSN-stimulated DCs. Secretion of IL-10 was increased in response to CSN and CSN-P, whereas, IL-12 was only increased in DCs stimulated with CSN-P. The percentages of apoptosis was increased in CSN treated DCs; conversely, CSN-P treatment significantly reduced apoptosis of DCs. Our results suggest that PGE2 had antagonistic effects to that of CSN, which reversed DCs into more mature, immunogenic and more resistant to apoptosis. These data may indicate a role for PGE2 as an immunomodulatory compound in anticancer immunity.
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1. Introduction

Dendritic cells (DCs) are the first that communicate with the invading pathogen that is threatening the host and their main duty is to present its antigens to other parts of the immune system and instruct different types of cells to eliminate this pathogen properly (Steinman, 2007). DCs are considered initiate the immune response and form a bridge to link between innate and adaptive immunity (Merad, *et al.*, 2013). In addition, DCs have great promises for the future of immunotherapy, which is a safer substitute for chemicals and drugs that has extensive side effect for the patient's health (Delamarre and Mellman., 2011). Cancer is considered the first leading cause of death threatening the human race (Jemal, *et al.*, 2011).

Cancer proliferation and spreading in the body means that it has found a way or even more than a way to evade the immune system and escape from being recognized, or perhaps take control of the immune system so that it work for the tumor's behalf (Talmadge and Gabrilovich, 2013). Interestingly, DCs were found in cancer environment lose their ability to alert the immune system (Bennaceur *et al.*, 2008). Some types of cancer have the ability to release different factors in their environment that contribute in altered maturation and apoptotic cell death of DCs (Kiertscher *et al.*, 2000). Apoptosis of DCs in the cancer environment significantly diminishes development of a specific antitumor immune response (Ma, *et al.*, 2013). Therefore, several research groups attempting to find the possible factors affecting DCs apoptosis in tumor microenvironment (Apetoh *et al.*, 2011).

A large body of evidence link between inflammation and cancer has generated intense interest in proinflammatory prostaglandins (Menter *et al.*, 2012). It has been documented that prostaglandin E2

(PGE2) is a potent regulator of the immune system (Kalinski., 2012). PGE2 is a soluble lipid compound driven by cyclooxygenase (Cox) Cox-1 and Cox-2 that can by produced by all cell types in the body, but majorly by epithelial cells, fibroblasts and inflammatory cells that highly control PGE2 production to prevent the damage of surrounding cells (Schmidt *et al.*, 2011). Moreover, inhibitors of the PGE2 biosynthetic enzyme cyclooxygenase 2 (COX-2) are used to treat pain and inflammation and are showing promise as cancer treatments (Lee *et al.,* 2009). Studies have demonstrated that PGE2 may either have a proapoptotic or antiapoptotic function depending on culturing condition and the origin and differentiation of cells under study (Lalier, *et al.*, 2011). Therefore, in the present study we aim to investigate the effects of PGE2 on human monocytes derived DCs. The generated DCs were also challenged with cancer cells supernatant (CSN) for comparing its effects with PGE2 alone or in combination (CSN-P) on the structure, phenotype, function and apoptosis of human monocytes derived DCs.

2. Materials and Methodology

2.1 Materials and preparations:

Dulbecco's Modified Eagle's Medium (DMEM), Roswell Park Memorial Institute 1640 (RPMI 1640) medium, Fetal calf serum (FCS), Penicillin-streptomycin solution, L-Glutamine solution, Trypsin 0.25% (1X) Solution, RBC Lyses buffer, PBS without Calcium Magnesium, and HBSS were purchased from HyClone (HyClone, South Logan, USA). Ethylene Diamine Tetra Acetic Acid (EDTA) (Sigma, St. Louis, USA). Lymphoprep™ sterile solution, was used for isolation of monocytes, with a density of 1.077 ± 0.001 g/mL purchased from Axis-Shield PoCAS, Norway. 16,16-Dimethyl Prostaglandin E2 (PGE2) was purchased from Tocris bioscience, Bristol, UK. A concentration of 5µg/mL was prepared by adding 9 mL of Dimethyle Sulfoxide (DMSO) to 1 mL of the 10mg/ mL soluble PGE2. Stock solution was sterilized by filtration and aliquoted in tightly sealed vials at -20°C. Purified LPS was purchased from Sigma Chemicals Co., St. Louis, MO and it is obtained from *Escherichia coli.* LPS stock was prepared in RPMI 1640 medium at a concentration of 1 µg/mL. Recombinant human (rh) GM-CSF and rhIL-4 were purchased from RnDsystem, Minneapolis, USA. Working concentrations of rhGM-CSF and rhIL-4 were prepared by dissolving 50 µg of rhGM-CSF and 100 µg of rhIL-4 in 1000 µL of RPMI 1640 medium to yield 800 U/ml and 500 U/ml respectively. Diluted cytokines were stored in 25 µL aliquots at -80°C for future experiments.

2.2. Generation of DCs:

DCs were generated as described by Romani et al. (1996). Briefly, 50 mL of blood were obtained from healthy donors and diluted in 50 mL of HBSS. Then, monocytes were isolated by slowly pouring 25 mL of diluted blood over 15 ml of Lymphoprep and obtaining density gradient centrifugation in 1400 rotation per minutes (rpm) for 30 minute without break at room temperature. Buffy coat was transferred into new falcon tubes and washed with HBSS and then centrifuged in 1600 rpm for 10 minutes at 4 °C. Cells were treated with 25 mL of RBC Lyses buffer and incubated for 10 minute at room temperature. Then cells were washed twice in HBSS, and were resuspended in 50 mL of HBSS. Trypan blue cell viability assay was determined using contrast phase-contrast inverted microscope (Nikon eclipse Ti, Tokyo, Japan) supplemented with digital camera software (NIS-Elements F 3.2, Nikon, Tokyo, Japan). A total of $50x10^6$ monocytes were resuspended in 18 ml of RPMI 1640 medium and seeded in a 12-well plate, with a concentration of approximately 5x105 cells mL-1 per well. After 90 minutes of incubation in 5% CO2 humidity at 37°C, non-adherent cells were gently removed by washing plates with RPMI-1640 media several times. The remaining adherent cells were then incubated in RPMI-1640 medium that supplemented with 800 U/mL of rhGM-CSF and 500 U/mL of rhIL-4 and incubated for 7 days. On day four, 200 µL of media was removed and replaced by fresh RPMI-1640 medium supplemented with rh GM-CSF and rh IL-4 for refeeding. DCs maturation was stimulated on seventh day by adding either 1 µg/mL of LPS, 5 µg/mL of PGE2, or replacing 500 µL of the DCs media with DCs conditioned media (CSN, or CSN-P) for 24 hours. DCs culture supernatants were removed for IL-10 and IL-12 ELISA test and DCs were washed with cold PBS, and were stained for flow cytometry examinations.

2.3. Culturing of breast cancer cell line:

A total number of $45x10^4$ cells of Michigan Cancer Foundation-7 (MCF-7) were seeded in each well of 6-well plate and incubated in DMEM for 24 hours in 5% CO_2 humidity at 37°C. On 75 % cell line confluence, 75µL of PGE2 (5 µg/mL) were added to some wells. After 24 hours of adding PGE2, cancer supernatant (CSN) from wells with PGE2 (CSN-P), and without PGE2 (CSN) were separated. All supernatants were centrifuged and filtered using 0.22 µm syringe filters to remove cells and debris and then were aliquoted and stored at -80°C for further experiments.

2.4. Flow cytometry analysis of DCs surface markers:

To determine DCs phenotype, cells were washed with cold PBS, resuspended in RPMI media and they were aliquoted in different tubes for staining with the following recombinant monoclonal human antibodies (MoAb): CD14, CD80, CD83, CD86, CD11c, HLA-

DR and IgG isotype control. All MoAb were purchased from R and D system, Minneapolis, USA. Following staining, cells were incubated on ice in a dark room for 30 minutes. Cells were then washed twice with PBS and finally resuspended in 500 µL of PBS for acquiring by flow cytometry.

2.5. DCs apoptosis:

2.5.1. Annexin V assay

Annexin V-FITC apoptosis detection kit was purchased from Trevigen, Gaithersburg, USA. Cells were washed once and resuspended in 100 µl of Annexin-V reagent. Cells were incubated in the dark for 15 minutes at room temperature. Finally, 400 µl of 1X binding buffer was added to samples (per 100 µl reaction). Ten µl of PI was added to cells prior analysis by flow cytometry.

2.5.2. Cell cycle analysis by flow cytometry

Cell cycle analysis was performed using a commercial kit (Coulter DNA Prep™ Reagents Kit; Beckman Coulter, Fullerton, CA, USA). Cells were plated in six-well plates and incubated overnight with different stimuli. Following treatment, cells were harvested, then washed twice with pre-chilled PBS and resuspended in 100 µl PBS at a concentration of 1×10^6 cells/ml. Each cell sample was mixed with 100 µl DNA Prep LPR (contained in Coulter DNA Prep™ Reagents Kit), gently mixed by vortex and incubated in the dark at room temperature (25°C) for 20 minutes. Then each was mixed with 1 ml of stain (DNA Prep Stain; contained in Coulter DNA Prep™ Reagents Kit), gently mixed by vortex and again incubated in the dark at room temperature (25°C) for 20 minutes. Finally, cell cycle analysis was performed within 1 hour using Navios flow cytometer and analyzed with the Navios software (Beckman Coulter, Villepinte, France).

2.6. Determination of cytokines:

The concentration of IL-10 and IL-12 in DCs supernatant were measured using enzyme-linked immunosorbent assay (ELISA) using Human IL-12 (p70) ELISA kit and Human IL-10 ELISA kit (Biolegend, San Diego, USA), according to the manufactures instructions.

2.7. Statistical analysis of data

All data were statistically analyzed using Microsoft Excel 2008 and Statistical Package for Social Sciences (SPSS). Data were expressed as means ± standard division (SD). *T-test* was performed to compare between mean of groups where *P*-value < 0.05 was considered as statistically significant results.

3. Results

3.1. DCs morphological characteristics:

DCs morphology was monitored during the differentiation of human peripheral blood monocyte using bright field inverted microscope (Figure 1. B-D). On day one, monocytes were appeared round in shape, small in size, and firmly adherent to tissue culture plate. On second day, the cells started to aggregate and form clusters (Figure 1. B, C). At the fourth day, cells changed to a typical morphology with irregular shape or veils (Figure 1. D). On 7th day, cells were stimulated with PGE2, CSN, and CSN-P for 24 hours. The following day, LPS-stimulated DCs cells showed irregular in shape with long veils as shown in (Figure 2. A); whereas PGE2, CSN, and CSN-P stimulated DCs become larger in size and show typical mature DCs shape expressing cytoplasmic veils (Figure 2. B-D).

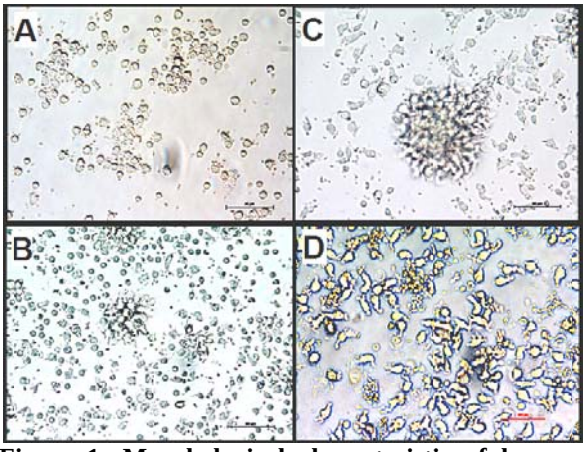

Figure 1: Morphological characteristic of human peripheral blood monocytes during differentiation into DCs. Cells on day one (A), cells forming clusters on day two and three (B, C), cells forming clusters of irregular shapes on day four (D). Original magnification was X400.

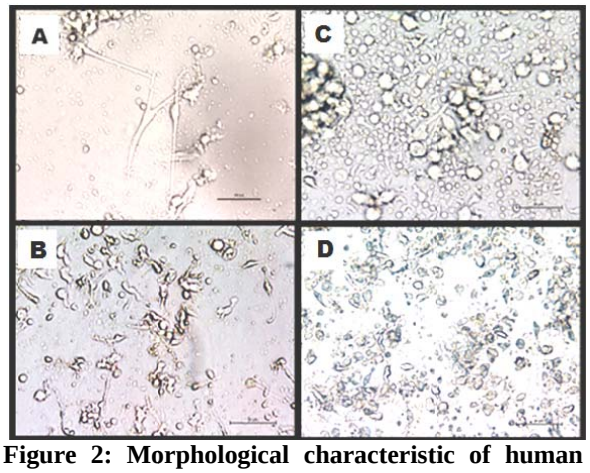

Figure 2: Morphological characteristic of human dendritic cells: Representative figures of DCs on day Eight. DCs were stimulated with LPS, PGE2, CSN, and CSN-P for 24 hours. LPS-stimulated DCs show typical mature DCs shape expressing long cytoplasmic veils (A), PGE2 stimulated DCs (B), CSN stimulated DCs

(C), CSN-P stimulated DCs (D). Original magnification was X400.

3.2 Dendritic cells immunophenotyping:

During maturation (stimulated by LPS), the expression of CD83, CD80 and CD86 was upregulated. The expression of costimualtion and maturation markers CD80, and CD83 were not increased in DCs stimulated with PGE2, whereas CD86 expression was increased. Moreover, the expression of CD86 and CD83 was not increased in DCs stimulated with CSN. CSN-P stimulated DCs showed increased CD80, CD86 and CD83 compared with PGE-stimulated DCs, and only CD83 and CD86 compared with CSN (Table 1).

3.3. Dendritic cells apoptosis:

3.3.1. Annexin-V assay:

The percentage of early apoptotic cells annexin-V^+PI^- (Figure 3) of unstimulated DCs was 17.98±5.09 whereas in LPS stimulated DCs culture the percentage was 37.9±17.77. On the other hand, PGE2, CSN, and CSN-P stimulated DCs expressed significantly high percentage of apoptotic cells in comparison to unstimulated DCs. The percentage of late apoptotic cells annexin-V^+PI^+ of unstimulated DCs was 7.63±2.27, of LPS stimulated DCs 13.4±5.33, of PGE2 stimulated DCs 11.93±2.07, and of CSN stimulated DCs 8.7±0.68. Stimulation with CSN-P showed slightly higher percentage of cells that undergo late apoptosis (Figures 4).

Table 1: Immunophenotyping of monocytes-derived DCs after stimulation with PGE2, CSN, and CSN-P:

Stimuli / Marker	Unstimulated	LPS	PGE2	CSN	CSN-P
HLA-DR	99.55±0.35	99.8±0.00	99.25±0.78	99.3±0.57	99.55±0.35
CD14	6.35±0.64	3.7±0.57[a]	4.75±1.06	7.05±1.48	6.6± 2.97
CD80	48.65±4.03	99.15±0.21[a]	33.05±5.44[b]	90.8±7.64[ac]	81±0.85[ab]
CD83	6.05±1.63	92.65±0.35[a]	4.85±1.06[b]	12.9±5.23[b]	48±0.28[abc]
CD86	13.85±1.2	97.1±3.11[a]	49.95±7.14[ab]	24.75±4.03[abc]	72.2±0.71[ab]
CD11c	96.5±0.85	95.65±5.16	86.6±1.56	98.5±0.57[c]	92.05±0.49[a]

Values represents mean (S.D.) of three independent experiments from different healthy donors. (a) Significant compared to unstimulated DCs, (b) significant compared to LPS stimulated DCs, (c) significant compared to PGE2 stimulated DC. *P-value* < 0.05 was considered significant.

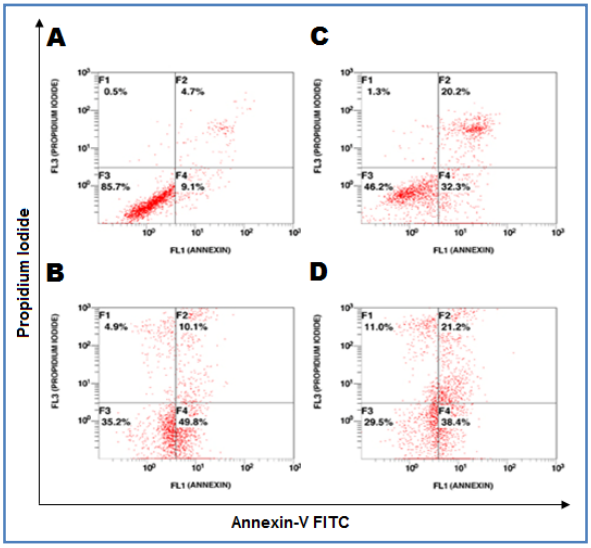

Figure 3: Representative dot blot for DCs apoptosis using Annexin-V assay after various stimuli. (A) unstimulated DCs, (B) stimulated with PGE2, (C) stimulated with CSN, (D) stimulated with CSN-P.

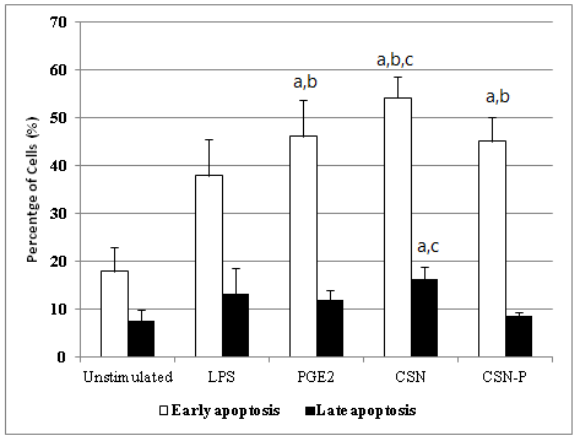

Figure 4: Percentages of DCs apoptosis determined by annexin-V after different stimuli. (a) Significant compared to unstimulated DCs, (b) significant compared to LPS stimulated DCs, (c) significant compared to PGE2 stimulated DC. *P-value* < 0.05 was considered significant.

3.3.2 Cell cycle Sub-G1 phase:

In cell cycle, cells that are found in the sub G_1 phase are considered apoptotic cells as due to low DNA staining. The percentage of apoptotic cells in the sub G_1 phase of unstimulated DCs was 6.43±1.43 and of LPS stimulated DCs was 8.94±3.37, PGE2 stimulated DCs was 12.81±3.89, CSN stimulated DCs was 14.38±0.30 and CSN-P was 10.18±2.72 (Figure 5).

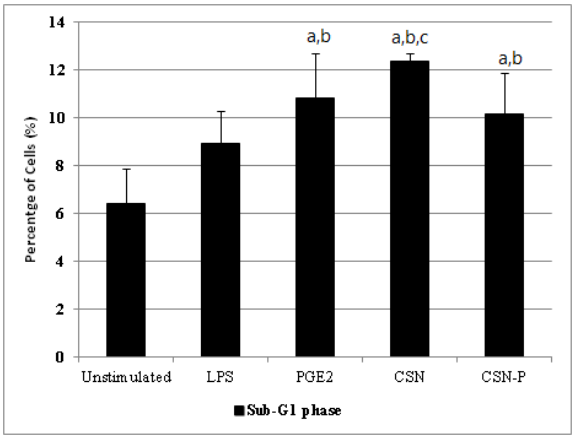

Figure 5: Percentages of DCs apoptosis determined by sub-G1 of cell cycle after different stimuli. (a) Significant compared to unstimulated DCs, (b) significant compared to LPS stimulated DCs, (c) significant compared to PGE2 stimulated DC. *P-value* < 0.05 was considered significant.

3.4. Cytokines in DCs cultures' supernatant:

The secretion of IL-10 by DCs stimulated with LPS was significantly high compared to unstimulated DCs. On the other hand, PGE2 stimulated DCs produced significantly low concentration of IL-10 in compare to LPS stimulated DCs. CSN-stimulated DCs showed significantly high levels of IL-10 in comparison to PGE2 stimulated DCs. On the other hand, IL-12 secretion by DCs in response to LPS was also significantly high compared to other cultures including unstimulated DCs. By contrast, PGE2 stimulated DCs was not different in IL-12 secretion from unstimulated DCs. CSN-stimulated DCs cultures showed slightly higher levels of IL-12. However IL-12 secretion was significantly higher in CSN-P stimulated DCs compared to unstimulated, and PGE2-stimulated DCs (Table 2).

Table 2: Levels of IL-10 and IL-12 secreted by monocytes-derived dendritic cells after stimulation with LPS, PGE2, CSN, and CSN-P

	Unstimulated DCs	LPS	PGE2	CSN	CSN-A
IL-10	**9.14±1.48**	**36.43 ±17.64[a]**	**8.51 ±3.82[b]**	**17.39±5.34[c]**	**17.86 ±4.08[c]**
IL-12	**30.16 ±16.22**	**640.47 ±230.5[a]**	**29.75 ±14.95[b]**	**33.81±9.1[b]**	**36.6±7.49[b,c]**

Values represents mean (±S.D.) of three independent experiments from different healthy donors. (a) Significant compared to unstimulated DCs, (b) significant compared to LPS stimulated DCs, (c) significant compared to PGE2 stimulated DC. *P-value* < 0.05 was considered significant.

4. Discussion

Since dendritic cells are not prevalent in the blood stream, a model whereby monocyte-derived dendritic cells is used widely as an experimental model to study human dendritic cells (Romani et al., 1996). In the present study, monocytes-derived DCs were used to study the impact of PGE2, cancer cells supernatant (CSN) and their combination (CSN-P) on DCs morphology, phenotype, and apoptosis.

We first studied the morphological properties, as it was reported that the morphology of DCs correlates to their level of maturity and activation (Granucci, et al., 1999; Jia, et al., 2012). DCs generated from monocytes started to show typical mature DCs morphology as they enlarged and form long cytoplasmic veils or dendrites, which are similar to that observed by Carrasco, *et al*., (2001) and Leon, *et al.,* (2004) studies. PGE2 and CSN stimulated DCs showed shorter veils and cells were in more rounded shape, which was also in agreement with previous reports (Landi, *et al.,* 2011; Koido, *et al.,* 2004). As for CSN-P stimulated DCs, they showed smaller irregular shapes with shorter dendrites.

DCs' phenotypical changes were then studied, as their functions in the immune system and the nature of signals they transduce to T lymphocytes were found dependant on their phenotype (Leon and Ardavin, 2008). In this study, we identified the phenotype of monocytes derived DCs using DC specific markers such as HLA-DR and CD11c on unstimulated DCs and DCs stimulated with LPS, PGE2, CSN, and CSN-P, and found that both markers are increased in unstimulated DCs and upon DCs stimulation with LPS, PGE2, CSN, or CSN-P, which prove the success of differentiation of monocytes to DCs. Moreover, expression of CD14, a monocyte specific marker, was decreased in all DCs cultures, which confirms the differentiation of $CD14^+$ monocytes into $CD14^-$ DCs. These data are in agreement with results of Romani, *et al*., (1996), and Li, *et al*., (2011).

The expression of the maturation marker CD83 was also investigated, as its expression on tumor infiltrating DCs has been proven to be important as

regard tumor relapse and survival rates in breast cancer patients (Iwamoto, *et al.*, 2003). Our flow cytometry results showed significantly higher expression of CD83 in LPS-stimulated DCs compared with unstimulated DCs, which indicates full maturation. These results are in agreement with data reported by Butler, *et al.*, (2007). However, CSN slightly affected the expression of the maturation marker CD83 on DCs, which was significantly low in comparison to LPS stimulated DCs. These results were similar to studies of Liu, *et al.*, (2010); Pei, *et al.*, (2014) in which they found that DCs stimulated with supernatant of pancreatic cell culture expressed low CD83 in comparison to DCs stimulated with cancer cells treated with chemotherapeutic agents. As regard PGE2, we detected low expression of CD83 on PGE2-stimulated DCs in comparison to unstimulated and to LPS-stimulated DCs. Interestingly, expression of CD83 on DCs after addition of CSN-P was significantly higher than that of unstimulated DCs, PGE2 alone or CSN-stimulated DCs alone. These data propose that PGE2 reversed the inhibitory effect of CSN alone on DCs maturation and derived DCs toward more mature phenotype and support the hypothesis that PGE2 may be considered as an immunological modifier that support immune response against cancer as was reported earlier by Lalier, *et al.*, in 2011.

We further studied the costimulatory molecules CD80 and CD86 that are used to indicate the functional properties of DCs in T-cell interactions and T-cell functional activation. Although CD86 can promote specific CTL immune response upon attachment to its ligand CD152 on T cell, CD80 regulate the CTL-CD152 pathway in order to tolerate immune response (Manzotti, *et al*., 2002; Zheng, *et al*., 2004). The effect of CSN alone on DCs promoted significantly the expression of CD80 in comparison to unstimulated DCs, whereas the expression of CD86 was not significantly different to that of unstimulated DCs. These findings suggest that CSN induced an immunosuppressive microenvironment to evade antitumor immune response. These data are consistent with previous report of Ma, *et al.*, in 2010, which demonstrated that culturing DCs with uveal melanoma supernatant downregulated expression of CD86 but not CD80 even after stimulating DCs with TNF-α for 24 hours. As regard the effect of PGE2 alone on DCs, we detected low expression of CD80, and increased expression of CD86 on DCs' surface in comparison to unstimulated DCs. These results are in agreement with previous studies (Rieser, *et al.*, 1997; Kalinski, *et al.*, 1997). Additionally, we found that treating DCs with combination of PGE2 and CSN for 24-hours (CSN-P) reduced further the expression of CD80 and increased the expression of CD86 on DCs in comparison to DCs treated with CSN alone. These findings suggest that PGE2 impact on DCs antagonized the inhibitory effects of CSN alone on DCs.

After confirming the maturation and phenotypical properties of DCs and their alterations under different conditions, we studied the cell death of DCs under the same conditions. Cancer supernatant used in this study (CSN) reduced DCs survival and increased their apoptosis significantly in comparison to unstimulated DCs, which is known as one of the cancer mechanism used to avoid DCs immunosurveillance and escape tumor elimination as previously suggested by Maier, *et al.*, in 2009. In addition, PGE2-treated DCs showed slightly higher percentages of apoptotic cells in comparison to unstimulated DCs. However, combination of PGE2 with CSN (CSN-P) induced lower percentage of apoptosis than CSN alone, indicating that PGE2 has protective effect against DCs' apoptosis-induced by CSN. These findings are in agreement with previous studies reported that PGE2 may confer protection against DCs cell death via expression of survivin; a member of the inhibitor of apoptosis (Baratelli, *et al.*, 2005).

Several studies reported that incubation of these DCs cells with CD40 or IL-12 caused the elevation of Bcl-2 expression on DCs, which accordingly prevented DCs apoptosis (Pinzon-cherry, *et al.*, 2005). Similarly, apoptosis was prevented in DCs co-cultured cancer when stimulated with IL-12 and IL-15 (Pirtskhalaishvili, *et al.*, 2000). Therefore, we investigated the concentrations of IL-12 as well as IL-10 in DCs' culture media after each stimulus to explore their associations with DCs' apoptosis. In our results, LPS individually, was able to significantly promote DCs to secrete elevated levels of IL-10 and IL-12p70 in comparison to unstimulated DCs. On the other hand, PGE2 alone reduced production of IL-10 or IL-12 from DCs, which is in accordance with the results of Baratelli, *et al.*, 2005 that used the same PGE2 concentration as in our study. Moreover, we did not detect significant alterations in IL-12 production from CSN stimulated DCs. However, CSN-P stimulated DCs were found to secrete significantly higher IL-12 than unstimulated and PGE2 stimulated-DCs. In the mean time, we found IL-10 production significantly higher in DCs' culture with CSN and CSN-P in comparison to PGE2 stimulated DCs. Similar result found by Gerlini, *et al.*, in (2004) where supernatant derived from primary cancer cell line from melanoma patients induces DCs to produce significant higher amount of IL-10. These data suggest that CSN convert DCs into more tolerogenic DCs by potentiating IL-10 secretion and inhibit IL-12 production, whereas addition of PGE2 to CSN, although it did not affect IL-10 secretion, raised DCs

IL-12 cytokine production, which considered the main cytokine in the development of antitumor immune response as previously suggested by Landi et al., (2011).

Conclusions

DCs are found dysfunctional and more susceptible to apoptosis in patients with breast cancer. Control of apoptosis is fundamental for DC antitumor activity. Our data provide evidences that soluble factors in cancer cell supernatant had negative impact on DCs maturation, costimulatory functions and apoptosis. However, we found that addition of PGE2 conferred maturation, and protection of DCs from apoptosis that was associated with enhanced secretions of IL-12. These data suggest that PGE2 had antagonistic effects to that of CSN, which reversed DCs into more mature, immunogenic and more resistant to apoptosis. Finally, this study points to a role for PGE2 as an immunomodulatory compound in anticancer immunity.

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