Myeloid and lymphoid dendritic cells in the peritoneal fluid of women with ovarian cancer

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Abstract

Purpose: The aim of the study was to estimate the myeloid and lymphoid subpopulation of dendritic cells (DCs) in the peritoneal fluid (PF) of women with ovarian tumors.

Material and methods: We studied 34 patients with serous cystadenocarcinoma and 29 women with serous cystadenoma. Dendritic cells were isolated from peritoneal fluid, stained with monoclonal antibodies anti-BDCA-1 and anti-BDCA-2 and estimated using flow cytometry.

Results: Peritoneal fluid myeloid DCs constituted 0.59% of mononuclear cells in patients with ovarian cancer and 7.2% in women with serous cystadenoma. Lymphoid DCs constituted 0.39% of PF mononuclears in women with ovarian cancer and 0.07% in patients with serous cystadenoma. The percentage of lymphoid DCs was higher in patients with ovarian cancer than in women with serous cystadenoma.

The BDCA-1/BDCA-2 DCs ratio in peritoneal fluid of patients with serous cystadenoma was significantly higher in comparison to ovarian cancer.

Conclusions: Decreased BDCA-1/BDCA-2 DCs ratio in patients with ovarian cancer may favour Th2 lymphocyte differentiation and/or induction of immunological tolerance.

Key words: dendritic cells, peritoneal fluid, ovarian cancer.

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Introduction

Ovarian cancer is the fifth most common cancer in women and it is responsible for 5% deaths of females and over 50% of deaths caused by cancer of the female genital tract. Prognosis of the disease depends on several factors, including tumor margin, vascular invasion, tumor grade and stage, expression of oncogenes, and estrogen and progesterone receptors. Another possible prognostic factor are immune cells infiltrating the tumor [1].

Dendritic cells are the most potent antigen-presenting cells (APC). Two distinct DCs subpopulations of myeloid and of lymphoid origin have been described in humans. Myeloid DCs are a major subpopulation of human blood DCs which are $CD4^+$, Lin^-, $CD11c^{bright}$, $CD123^{dim}$, $CD45RO^+$ and $CD2^+$. They express myeloid markers (CD13, CD33) as well as Fc receptors (CD32, CD64, FcεRI). Myeloid DCs (DC-1) also express the BDCA-1 (CD1c) antigen which is characteristic for immature peripheral blood (PB) myeloid DCs [2,3].

Lymphoid dendritic cells (DC-2) have been described recently in human PB and lymphoid tissue. DC-2 cells express a specific BDCA-2 marker. Immunophenotyping of $BDCA\text{-}2^+$ dendritic cells characterises these cells as being $CD4^+$, Lin^-, $CD11c^-$, $CD123^{bright}$, $CD45RA^+$, $CD2^-$ and expressing neither myeloid lineage markers nor Fc receptors [3]. According to the myeloid or lymphoid origin DCs differ not only in immunophenotype but also in morphology and function. For the problem of anti-tumor immune response it could be important that myeloid DCs prime Th1 response as opposite to lymphoid DCs which prime Th2 response and tolerance [2,4].

The purpose of our study was to determine whether there are alterations of the DCs cells subsets and in the BDCA-1/BDCA-2 dendritic cell ratio in the peritoneal fluid between women with non-malignant and malignant ovarian tumors.

Material and methods

Of 59 women, aged 16-85 years, 34 patients were found to have FIGO stage III ovarian epithelial cancer with mean levels of Ca-125 marker 1853.97. No one women had neoadjuvant chemotherapy. Twenty nine women with benign tumors of ovaries were assigned to the reference group. The study was approved by the Lublin University School of Medicine Ethics Committee.

PF was aspirated from the anterior and posterior cul-de-sacs and taken into heparinized tubes. Mononuclear cells were isolated by density gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway). The cell surface antigens were determined on fresh cells at the time of a sample submission.

Isolated cells (1 x 10^6) were incubated for 20 minutes at 4°C with monoclonal antibodies (Moabs) specified against DCs surface antigens. The following monoclonal antibodies were used: anti-BDCA-1 (CD1c) FITC, anti-BDCA-2 FITC (Miltenyi Biotec) and anti-CD19 CyChrome, anti-CD123 PE (Pharmingen). Flow cytometric analysis of stained samples was performed on FacsCanto instrument (Becton Dickinson). A total of 300000 events were acquired and analysed using FacsDiva software. Cell debris and dead cells were excluded from the analysis based on scatter signals.

BDCA-1(CD1c) marker is expressed on a subpopulation of $CD19^+$ small resting B lymphocytes. The mononuclear cell analysis region was analysed for BDCA-1 (CD1c) and CD19 staining. BDCA-1 ($CD1c^+$) B cells were excluded from $CD1c^+$ peritoneal DCs by counter-staining for CD19. BDCA-1 ($CD1c^+$) $CD19^-$ cells were counted as immature myeloid DCs. Next, the mononuclear cell analysis region was analysed for BDCA-2 and CD123 antigens. BDCA-2^+CD123^+ cells are counted as lymphoid DCs (*Fig. 1*).

The results were expressed as a median and ranges. Statistical differences between groups were estimated using a standard non-parametric test (Mann-Whitney U test). P value less than 0.05 was considered as statistically significant.

Results

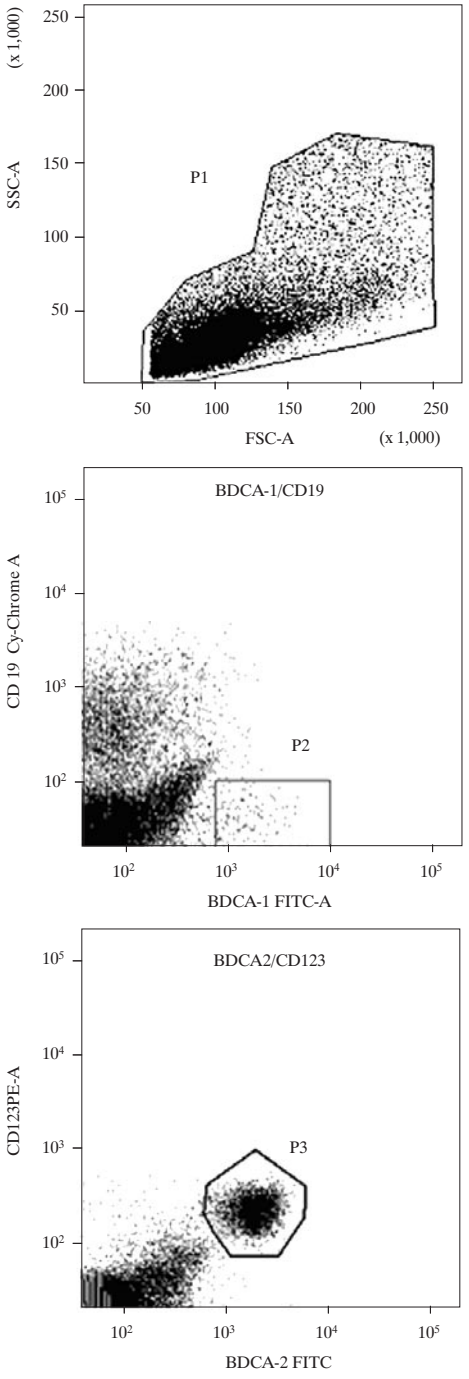
Both immature myeloid and lymphoid dendritic cells are detectable in all samples of PF of women with ovarian tumors (*Tab. 1*). Significantly higher percentages of myeloid in compared to lymphoid DCs in PF of women with serous cystadenoma were detected. There were no difference between the percentages of myeloid and lymphoid DCs in women with ovarian cancer.

The percentage of mononuclear cells that were identified as myeloid DCs was significantly higher (7.2% vs 0.59%) in women with non-malignant ovarian tumors in comparison to patients with ovarian cancer.

Lymphoid DCs constituted 0.39% of mononuclear cells in women with ovarian cancer and 0.07% in patients with serous cystadenoma. The percentage of lymphoid DCs was higher in patients with ovarian cancer than in women with serous cystadenoma (*Tab. 1*).

Significantly higher myeloid/lymphoid DCs ratio in PF of patients with non-malignant ovarian tumors (118.94) in comparison with ovarian cancer patients (1.41) was found.

Figure 1. **The identification of PF dendritic cells by flow cytometry region. The mononuclear cell analysis region (P1)applied to light scatters. The P1 gated events were analysed for BDCA-1 (CD1c) and CD19 staining and BDCA-1^+CD19^- cells were counted as immature myeloid DCs (P2). The P1 gated events were then analysed for BDCA-2 and CD123, and BDCA-2^+CD123^+ cells were counted as lymphoid DCs (P3)**



Discussion

Women with advanced ovarian cancer have often been shown to progressively develop impaired immune responses against autologous tumor cells, preceding the development of a more generalized state of immunosuppression [5]. Different

Table 1. **The percentages (% of mononuclears) of myeloid and lymphoid DCs in peritoneal fluid (PF) of patients with ovarian cancer and serous cystadenoma**

Group of patients	BDCA1$^+$/CD19$^-$ (%)			BDCA2$^+$/CD123$^+$ (%)			The BDCA-1/ BDCA-2 ratio
	Median	Min	Max	Median	Min	Max	
Cyst Adenocarcinoma	0.59	0.02	4.32	0.39	0.03	4.47	1.41
Serous Cystadenoma	7.2	0.62	24.48	0.07	0.01	0.43	118.94

mechanisms responsible for this phenomenon were described: such as generation of tumor-induced suppressor cells, alternations in T lymphocytes signal transduction, tumor induction of specific T cells apoptosis and development of peripheral tolerance to cancer's antigens [6,7].

DCs are the most potent antigen-presenting cells playing a key role in the induction of protective immune responses and maintenance of immunological memory. Their exceptional ability to instruct naïve T cells to initiate immune responses is critically beneficial for the host defence against neoplastic cells [2]. Furthermore, a large body of literature demonstrates a close relationship between the presence of DCs within various malignant tumors and prognosis. Thus, it was confirmed that DCs infiltration of solid tumors correlates with a better prognosis in head and neck tumors [8], melanoma [9], uterine cervical carcinoma [10] and ovarian cancer [1]. On the other hand it has been reported that in human suffering from cancer subpopulations of DCs are dysfunctional and consist of the phenotype immature DCs [7,11]. Several recent studies showed a significant decrease in the proportion and absolute numbers of DCs in peripheral blood [11,12]. The decrease of DCs in peripheral blood from cancer patients closely correlated with the stage and duration of the disease [11].

In our research we have made an effort to estimate the immature myeloid and lymphoid dendritic cells in PF and to investigate quantitative differences in subpopulations of DCs in women with ovarian cancer in comparison to DCs in non-malignant ovarian tumors.

We found that the percentage of myeloid DCs was significantly higher in the group of non-malignant ovarian tumor in comparison to patients with ovarian cancer. These results suggest that PF myeloid DCs population may be affected by the presence of the malignant disease and might contribute to diminished acquired immune responses observed in these women. In contrary, the percentage of lymphoid DCs was significantly higher in patients with ovarian cancer than in the reference group. This fact may be important for understanding of the mechanism of tumor immune escape, because lymphoid DCs are expected to induce tolerance rather than immunity. In study by Zou et. al. [4] it was shown that lymphoid DCs infiltrating ovarian carcinoma inhibited tumor-specific immunity by suppressing T cell activation. The investigation of Curiel et al. [13] shows comparable results in tumor ascites of women with ovarian carcinomas. They demonstrated that numerous functional lymphoid DCs accumulate in tumor ascites and inhibit antitumor immunity. The same authors found that lymphoid DCs produce high levels of the angiogenic cytokines (TNF-α and IL-8) and induce potent neovascularization in vivo. Thus, tumors may manipulate DCs distribution and alter DCs function to support tumor angiogenesis.

Other studies documented a significant dysfunction of type 1 T cell responses in tumor-bearing hosts, suggesting that tumor progression might be associated with a preferential type 2 T cell response [5]. However, factors which influence the Th2 predominance in tumor patients still remain enigmatic. The available data indicate that BDCA-1 and BDCA-2 DCs were claimed to stimulate Th1 and Th2 types of immune responses, respectively [2,3]. In our study we have demonstrated a significant accumulation of immature lymphoid DCs in the ascites of ovarian cancer patients. These cells may actively suppress the Ag-specific T cell response and thus could be involved in immunosuppression. Therefore, we concluded that PF lymphoid DCs in patients with ovarian cancer may favour Th2 lymphocyte differentiation and/or the induction of immunological tolerance which is now considerate one of the important mechanisms of tumor escape from immune system control.

The knowledge about DCs function and standardization of DCs culture conditions might represent a useful tool for cancer immunotherapy.

A further studies of the DCs function are necessary for complete understanding the influence of tumor microenvironment on DCs.

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References

1. Eisenthal A, Polyvkin N, Bramante-Schreiber L, Misonznik F, Hassner A, Lifschitz-Mercer B. Expression of dendritic cells in ovarian tumors correlates with clinical outcome in patients with ovarian cancer. Hum Pathol, 2001; 32: 803-7.

2. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. Annu Rev Immunol, 2000; 18: 767-811.

3. Dzionek A, Fuchs A, Schmidt P, Cremer S, Zysk M, Miltenyi S, Buck DW, Schmitz J. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. J Immunol, 2000; 165: 6037-46.

4. Zou W, Machelon V, Coulomb-L'Hermin A, Borvak J, Nome F, Isaeva T, Wei S, Krzysiek R, Durand-Gasselin I, Gordon A, Pustilnik T, Curiel DT, Galanaud P, Capron F, Emilie D, Curiel TJ. Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. Nat Med, 2001; 7: 1339-46

5. Santin AD, Bellone S, Palmieri M, Bossini B, Cane S, Bignotti E, Roman JJ, Cannon MJ, Pecorelli S. Restoration of tumor specific human

leukocyte antigens class I-restricted cytotoxicity by dendritic cell stimulation of tumor infiltrating lymphocytes in patients with advanced ovarian cancer. Int J Gynecol Cancer, 2004; 14: 64-76.

6. Niehans GA, Brunner T, Frizelle SP, Liston JC, Salerno CT, Knapp DJ, Green DR, Kratzke RA. Human lung carcinomas express Fas ligand. Cancer Res, 1997; 157: 1007-12.

7. Staveley-O'Carroll K, Sotomayor E, Montgomery J, Borrello I, Hwang L, Fein S, Pardoll D, Levitsky H. Induction of antigen-specific T cell anergy: An early event in the course of tumor progression. Proc Natl Acad Sci USA, 1998; 95: 1178-83.

8. Wischatta M, Sprinzl GM, Gunkel AR, Hussl B, Romani N, Schrott-Fischer A. Dendritic cells in selected head and neck tumors. Ann Otol Rhinol Laryngol, 2000; 109: 56-62.

9. Zehntner S, Townsend W, Parkes J, Schmidt C, Down M, Bell J, Mulligan R, O'Rourke M, Ellem K, Thomas R. Tumor metastasis biopsy as a surrogate marker of response to melanoma immunotherapy. Pathology, 1999; 31: 116-22.

10. Bethwaite PB, Holloway LJ, Thornton A, Delahunt B. Infiltration by immunocompetent cells in early stage invasive carcinoma of the uterine cervix: a prognostic study. Pathology, 1996; 28: 321-7.

11. Almand B, Resser JR, Lindman B, Nadaf S, Clark JI, Kwon ED, Carbone DP, Gabrilovich DI. Clinical significance of defective dendritic cell differentiation in cancer. Clin Cancer Res, 2000; 6: 1755-66.

12. Wojas K, Tabarkiewicz J, Jankiewicz M, Rolinski J. Dendritic cells in peripheral blood of patients with breast and lung cancer – a pilot study. Folia Histochem Cytobiol, 2004; 42: 45-8.

13. Curiel TJ, Cheng P, Mottram P, Alvarez X, Moons L, Evdemon-Hogan M, Wei S, Zou L, Kryczek I, Hoyle G, Lackner A, Carmeliet P, Zou W. Dendritic cells subsets differentially regulate angiogenesis in human ovarian cancer. Cancer Res, 2004; 64: 5535-8.