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ARTICLE

Immunophenotyping of Tumor-Infiltrating Mononuclear Cells in Ovarian Carcinoma

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Infiltrating mononuclear cells play an important role in many types of cancer. The aim of this work was to determine the immunologic characteristics of mononuclear cellular infiltrate in ovarian cancer as compared to benign ovarian tumors. Paraffin-embedded tissues obtained from 52 ovarian carcinomas and 21 benign ovarian neoplasms were examined immunohistochemically to demonstrate suppressor/cytotoxic T cells and macrophages by using CD8 and CD68 monoclonal antibodies, respectively. The mean percentage of CD8+ cells was much higher in the malignant than in the benign group (P=0.00009). Similarly, the mean level of CD68+ cells was significantly higher in carcinomas than in benign cases (P=0.006). There was a significant negative correlation between the percentage of CD8+ cells and CD68+ cells in the malignant group (P=0.000002). Conversely, no correlation could be obtained between the values of these two cell types in the benign lesions. In the malignant group, although the percentages of CD8+ cells and CD68+ cells were not related to tumor differentiation, they were significantly related to tumor type. CD8+ cells were significantly higher in the serous (P=0.02), and CD68+ cells were higher in the mucinous carcinomas (P=0.0005). CD8+ T cells and macrophages constitute a major component of the infiltrating mononuclear cells in ovarian carcinoma. Their frequency seems to be related to the tumor type rather than the degree of tumor differentiation. (Pathology Oncology Research Vol 10, No 2, 80–84)

Keywords: Ovarian cancer, benign ovarian tumors, CD8+ cells, CD68+ cells

Introduction

Tumor-infiltrating mononuclear cells indicate an active immune response of the host that may be directed against the tumor. In certain tumors including ovarian tumors, the presence of these cells in high numbers is associated with a more favorable prognosis and prolonged survival.[1] Tumor-infiltrating mononuclear cells consist of T lymphocytes (helper and suppressor/cytotoxic), natural killer cells, B lymphocytes, and macrophages.[2]

Most of the T-cell infiltrate consists of CD8+ cells which can mediate specific cytotoxicity against tumor cells.[3] On the other hand, macrophages (CD68+ cells) represent a major component of the tumor-infiltrating mononuclear cells. Their primary role is to recruit and activate lymphocytes through the presentation of tumor antigens.[4] Nevertheless, their functional heterogeneity may result in antagonistic functions. Thus, they can inhibit the proliferation of some tumor cells, while simultaneously supporting the growth of others.[5]

In order to elucidate the role of some tumor-infiltrating mononuclear cells in epithelial malignant tumors of the ovary, as compared to nonmalignant lesions, we have conducted an immunohistochemical study for the determination of CD8+ and CD68+ cells in 52 ovarian carcinomas and 21 benign ovarian tumors.

Materials and methods

Specimen collection and preparation

From the files of the Pathology Department, Ain Shams Faculty of Medicine, we selected the cases of ovarian carcinomas processed during the period of January 2000 to December 2000. As a control group, benign epithelial ovarian tumors processed during the same period were also selected. Samples (benign as well as malignant) from patients who had other diseases, or received medication that could affect the mononuclear cells, were excluded.

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For all ovarian tumors included in the study, paraffin blocks were retrieved and used for preparation of hematoxylin and eosin (H&E)-stained tissue sections. The latter were examined to confirm the histologic diagnosis to determine the tumor type and grade in the malignant group according to Scully et al.[6]

Immunohistochemistry

Four-micron-thick sections, cut from paraffin blocks of the ovarian tissues, were used for the immunohistochemical studies. The tissue sections were immunostained for demonstration of suppressor/cytotoxic T cells and macrophages by applying anti-CD8 and anti-CD68 mouse monoclonal antibodies, respectively (Dako, Denmark). Both antibodies were available ready to use. The immunohistochemical technique was performed by using the supersensitive streptavidin-biotin detection system (Biogenex, CA, USA), according to Hsu and Raine.[7] Briefly, the tissue sections were deparaffinized in xylene and descending grades of alcohol. After blocking the endogenous peroxidase activity, retrieval of the antigen was performed by treating the slides in 10 mM citrate buffer, pH 6.0 in a microwave oven for 5 min. Then, the primary antibodies (CD8 and CD68) were added to the tissue sections and incubated for one hour at room temperature. After that, the slides were incubated with the secondary biotinylated antibody (goat anti-mouse IgG) and streptavidin-peroxidase, each for 30 min. Antibody binding was visualized by adding diaminobenzidine (DAB) as a chromogen and hematoxylin for counterstaining. Slides of a tonsil were used as positive control for both antibodies. For each case, negative control was applied by replacing the antibody by PBS or non-immune serum. For each cell population, 200 cells were counted and the positively immunostained ones were expressed as percentages of all mononuclear cells[8]. The slides were reviewed without knowledge of the case-control status of the study subjects.

Statistical analysis

The correlation between CD8+ and CD68+ cell populations was determined by using Spearman's rank correlation test. Comparison of the mean cell populations between the benign and malignant groups was performed with Student's t-test. Both cell populations were correlated to the tumor type and grade by applying Student's t-test and F-test, respectively.

Results

Histologic findings

Fifty-two malignant ovarian neoplasms were included in the study. They consisted of 28 serous and 24 mucinous cystadenocarcinomas, graded as follows: grade I (12), grade II (18) and grade III (22). The control group included 21 benign ovarian tumors (11 serous and 10 mucinous cystadenomas). The inflammatory mononuclear cells were present diffusely between the tumor cells and in the tumor stroma.

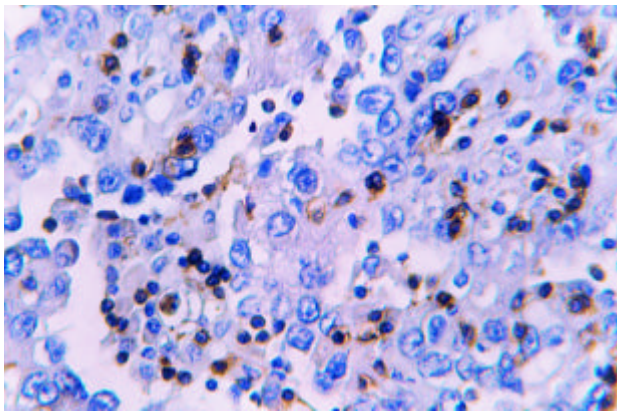

Figure 1. *A case of ovarian adenocarcinoma showing infiltration by CD8+ cells, immunoperoxidase X400.*

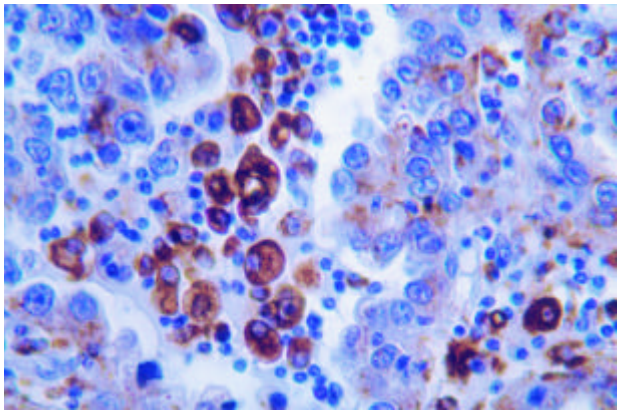

Figure 2. *A case of ovarian adenocarcinoma showing infiltration by CD68+ cells, immunoperoxidase X400.*

Immunohistochemical findings

CD8+ cells were observed in all malignant tumors. The positive immune reaction was highlighted by the brownish cytoplasmic staining (*Figure 1*). The percentage of CD8+ cell population in the malignant group varied from 10% to 60% (mean 24.2%). However, in the benign group CD8+ cells were seen in 6 cases only (28.6%) with a percentage varied from 2% to 50% (mean 7.2%).

Similarly, all cancer cases contained CD68+ cells showing brownish granular cytoplasmic staining (*Figure 2*). The percentage of this cell population varied from 10% to 80% (mean 51.2%). In the benign group, CD68+ cells were seen in 12 tumors (57. 1%) with a ratio ranging between 5% and 70% (mean 32.9%).

Statistical correlation

There was a highly significant negative correlation between the percentage of CD8+ cells and CD68+ cells in the malignant group, i.e. the increase in the level of CD8+ cells was associated with a decrease in the level of-CD68+ cells (Spearman's rho p=0.59, P=0.000002) (*Figure 3*). Conversely, the benign group showed positive correlation between the two cell populations. Yet, this relationship was of borderline significance (Spearman's rho = 0.44, P=0.044) (*Figure 4*).

The mean level of CD8+ cells was significantly higher in the malignant than in the benign group (t=4.14, P=0.00009). Also, the mean level of CD68+ cells was significantly higher in the malignant than in the benign cases (t=2.8, P=0.006).

The percentage of neither CD8+ cells nor CD68+ cells was related to the grade of tumor differentiation in the ovarian carcinomas (*Table 1*). However, both cell populations were related to the histologic tumor type. The ratio of CD8+ cells was significantly higher in the serous than in mucinous carcinomas (t=2.32, P=0.02). Conversely, the ratio of CD68+ cells was significantly higher in the mucinous than in serous carcinomas (t=3.74, P=0.0005) (*Table 2*).

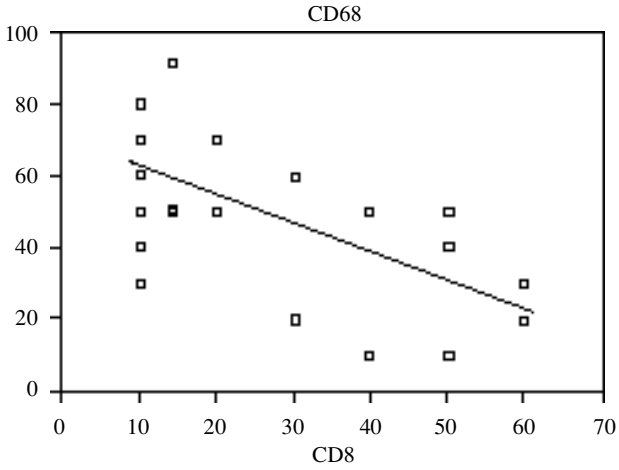

Figure 3. *Negative correlation between CD8+ cells and CD68+ cells in the malignant group (Spearman's rho correlation, n= -0.59, P=0.000002)*

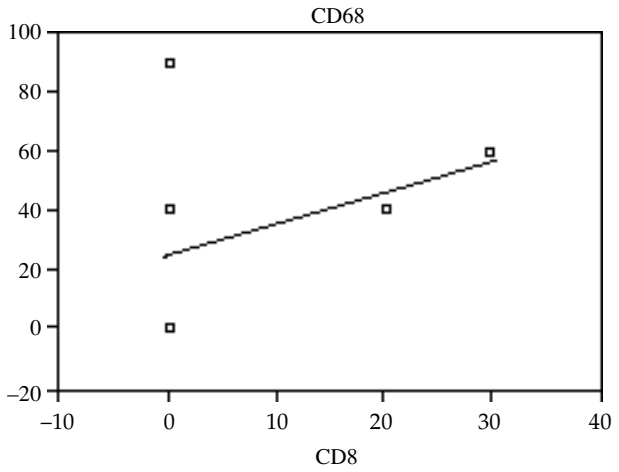

Figure 4. *Positive correlation between CD8+ cells and CD68+ cells in the benign group (Spearman's rho correlation, n=0.44, P=0.04)*

Discussion

The host-tumor interaction may play an important role in determining tumor progress. Recent studies have shown that this interaction can be influenced by the release of soluble factors by tumor cells and tumor-infiltrating lymphocytes (TIL).[9] Early studies performed by Chen et al.[10] applied a leukocyte migration test to detect the cell-mediated immunity in patients with ovarian carcinoma. Later on, the nature of the inflammatory cells in ovarian tumors was evaluated by Kabawat et al.,[11] who suggested that the in situ immune response to ovarian cancer is likely to be T-cell dependent.

The aim of the present study was to investigate, in part, the immunologic characteristics of the mononuclear cellular infiltrate in ovarian cancer as compared to benign ovarian tumors, in order to clarify the tumor-host relationship.

We have found that macrophages (CD68+ cells) constitute about 51% of the infiltrating mononuclear cells in ovarian carcinomas. This finding is consistent with previous reports.[4,12] However, Mergoni et al[13] obtained much lower values. It is worth mentioning that mucinous cystadenocarcinomas included in our study revealed a significantly higher percentage of macrophages than serous cystadenocarcinomas. Therefore, the lower percentage of macrophages, reported in the study of Mergoni et al., may be due to the fact that most of their cases (12 out of 16) were serous cystadenocarcinomas. Nevertheless, the methodological variation may also account for the different data obtained in the current study and that of Mergoni et al.[13]

By comparing benign and malignant ovarian tumors, we have found that both suppressor T cells and macro-

Table 1. **Relationship between the percentage of mononuclear cells and grades of ovarian carcinomas**

Cell type	*Tumor grade (N)*				
(Mean ± SD)	*I (12)*	*II (18)*	*III (22)*	F	P
CD8+ cells	21.70 ± 17.50	27.80 ± 22.10	22.70 ± 12.40	0.58	NS
CD68+ cells	40.00 ± 17.01	55.60 ± 20.10	53.60 ± 21.50	2,45	NS

NS: not significant

Table 2. **Relationship between percentages of mononuclear cells and types of ovarian carcinomas**

Cell type (*Mean ± SD*)	*Tumor Type (N)*		t	P
	Serous (24)	*Mucinous (28)*		
CD8+ cells	30.00 ± 21.30	19.30 ± 11.20	2.32	0.02
CD68+ cells	40.80 ± 19.30	60.00 ± 17.60	3.74	0.0005

phages were significantly higher in the malignant than in benign neoplasms (P=0.00009, P=0.006 respectively). This result is supported by the data of Mantovani et al.[14] and Zusman et al.,[15] who concluded that both T cells and macrophages play an important role in the pathophysiology of ovarian cancer. Similar findings have been obtained by Kabawat et al.[11] who attributed the higher ratio of lymphocytes in ovarian carcinomas to the better recognition of malignant tumors by the immune system. Alternative explanations may lie in the frequent occurrence of necrosis of malignant tumor cells with subsequent release of chemotactic factors. However, Kullander and Rausing[16] reported that both T cells and macrophages were sparsely represented in benign and malignant ovarian tumors. Accordingly, they concluded that immunogenic activity would be weak in ovarian cancer, a concept that contrasts with our findings and those of others.[14-16]

Correlation between the ratios of suppressor T cells and macrophages in the malignant cases revealed a highly significant negative relationship (P=0.000002). This finding is possibly due to secretion of oxides such as hydrogen peroxidase by macrophages, which inhibits the proliferative response of T lymphocytes.[17] On the other hand, Negus et al.[4] found no correlation between T cells and macrophages in cases of ovarian carcinoma. Such variations may be explained by the diverse functions of macrophages that can influence the response of the other infiltrating mononuclear cells.

Very few studies investigated the relationship between the amount of tumor-infiltrating mononuclear cells and the histologic grade of ovarian carcinoma. Negus et al.[4] as well as the current study showed no correlation between the ratio of CD8+ or CD68+ cells and the tumor grade. These data may suggest that the degree of tumor differentiation does not influence the degree of immune response of the patient. Lack of relationship between the ratio of tumoral infiltration with mononuclear cells and the histologic grade of the tumor was also reported in renal cell carcinoma[18] and colorectal carcinoma.[19] However, Puccetti et al. found an association between the nuclear grading of the neoplastic cells and the local tumor specific T cell responses in renal cell carcinoma.[20]

In conclusion, our study revealed that cytotoxic cells and macrophages constitute a major component of tumor-infiltrating mononuclear cells in ovarian cancer. The higher ratio of these cells in malignant as compared to benign ovarian tumors suggest that they have a significant role in the pathogenesis and/or pathophysiology of ovarian cancer. We have also concluded that the percentage of these tumor-infiltrating mononuclear cell populations is possibly influenced by the histologic type of the tumor rather than the grade of tumor differentiation.

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