Immune Effect of Tumor-infiltrating Lymphocytes and Its Relation to the Survival Rate of Patients with Ovarian Malignancies

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Summary: Tumor-infiltrating lymphocytes (TIL) in histological sections from 94 patients with ovarian malignancies were quantitated for their relation to the prognosis of the patients. It was found that the degree of lymphocyte infiltration directly affected the patients' survival rate and also related to clinical staging of the patients, as well as grading and histologic typing of the tumor. T lymphocytes characterized by alpha-naphthyl acetate (ANAE) staining were the major cells in the stroma of ovarian malignancies. Ultrastructural study of TIL and cancer cells suggested that tumor cell degeneration is related to the effect of TIL.

Key words: ovarian malignancy, tumor-infiltrating lymphocytes, prognosis, ultrastructure

Ovarian malignancy is the leading cause of death in patients with gynecologic cancer. The prognosis depends on a multitude of factors including clinical staging of the patients, cell grading and histologic typing of the tumor, residual tumor burden and systemic immune status of the host. The possible relevance of tumor-infiltrating lymphocytes (TIL) in stroma to the prognosis of patients is so far poorly understood. In view of the possible role of TIL in the growth control of tumor, we planned in this study to evaluate the effect of TIL on the patients' survival rate and the probable relationship between TIL and tumor cell lysis.

MATERIALS AND METHODS

Subjects

HE stained sections from 94 specimens of ovarian cancers surgically removed from patients admitted to our hospital from 1982 to 1988, were investigated. These cases included 47 of epithelial cancer, 20 of germ cell tumor, 17 of sex cord stromal tumor and 9 of ovarian metastatic tumor. Frozen sections and alpha-naphthyl acetate esterase (ANAE) staining were made for tumor specimens from 15 cases. Tumor specimens from other 4 cases were subjected to electron microscopic observation.

Observation on HE stained sections

The number of TIL within the tumor cell-normal stroma interface was examined (fig. 1). TIL in each of the 10 randomly sampled high power fields were counted, and their number was expressed by the mean value ($\bar{x}$). Lymphocyte infiltration was graded as being mild ($\bar{x}<5$); moderate ($5\leqslant\bar{x}\leqslant 15$) and marked ($\bar{x}>15$), respectively.

ANAE staining

The tumor tissues were fixed for

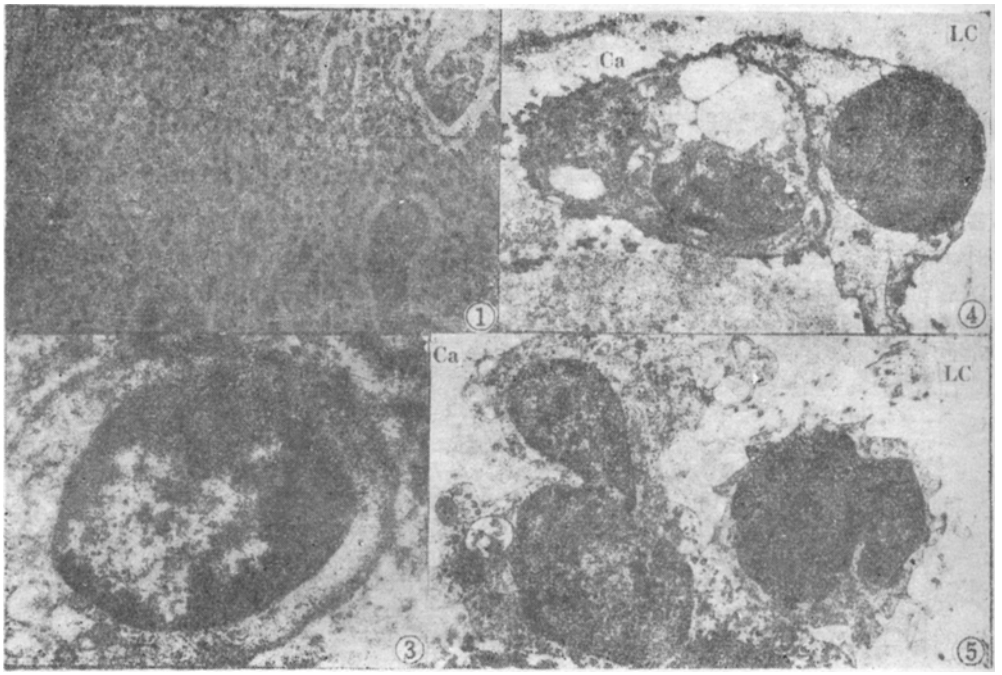

Fig. 1. HE staining: ovarian serous cystadenocarcinoma (3.5×20). Marked diffuse TIL in the proximity of tumor tissues.

Fig. 3. Normal ovary specimen. Normal lymphocytes with nuclear hyperchromatosis, few organelles. 8000×.

Fig. 4. Ovarian cystadenocarcinoma. Lymphocytes in contact with cancer cell, have a lot of vial processes on the cell membrane and a hyperchromatic nucleus with deep notch. Cancer cells appear to have undergone degeneration. 6500×.

Fig. 5. Ovarian matastatic cancer. Lymphocytes in contact with cancer cells. Cancer cells exhibit signs of injury including vacuolization and cytoplasmic swelling. 6300×.

24 h at 4℃ in 10% neutral formalin calcium solution and for another 24 h at 4℃ in Holt's solution. The tissues were then washed in running tap water for 10 min. Frozen sections were made (5 μm thick) with the AO frozen section machine, and immediately stuck onto slides. These sections were incubated for 2 h at 37℃ in a culture medium containing freshly prepared solution of hexazonium and alpha-naphthyl acetate (pH 5.9-6.1). The slides were rinsed to be freed from visible deposits and counterstained in the solution of methyl green for 30 min, rinsed again and dried. At least 100-200 lymphocytes were counted under the oil immersion objective. Cells with one or several distinct gayly-coloured brown-red dots or spots in the cytoplasm were scored as positive.

Electron microscopic examination

Fresh tumor tissues sampled within 5 min after cut-off of blood supply were put immediately into the solution of 2.5% glutaraldehyde and fixed at 4℃ for 2 h, and then rinsed three times in 7% sucrose solution. The tissues were then subjected to further fixing, embedding and ultramicrotomy, successively. The carcinolytic effect of TIL on tumor cells was examined under the transmission electron microscope.

RESULTS

1. **The degrees of tumor lymphocyte infiltration**

1) Clinical staging: The pair wise difference in the degree of tumor lymphocyte infiltration between stages Ⅰ and Ⅱ, Ⅳ was highly significant (table 1; $P<0.01$). Similarly, the difference between the stages Ⅱ and Ⅲ, Ⅳ was also significant ($P<0.05$). The difference in TIL between Ⅰ and Ⅱ, as well as between stages Ⅲ and Ⅳ, however, was insignificant. These results showed evidence of abundant TIL in the stroma of early-stage neoplasm.

2) Tumor cell grading: In the 94 cases studied, there were 47 of epithelial ovarian cancer. The degree of tumor lymphocyte infiltration in cases of grade Ⅰ was much higher than in those of grades Ⅱ and Ⅲ, the difference being of statistical significance ($P<0.05$), but the difference between cases of grades Ⅱ and Ⅲ, was insignificant (table 2; $P>0.05$). These results suggest that cases with well differentiated tumor cells have high degree of tumor lymphocyte infiltration.

3) Histologic typing: We investigated 47 cases of epithelial cancer, 21 cases of germ cell tumor, 17 cases of sex cord stromal tumor and 9 cases of ovarian metastatic tumor. The TIL number in the group of germ cell tumor was obviously greater than those in other groups, the difference being of statistical significance ($P<0.01$). The difference among the groups of epithelial cancer, sex cord stromal tumor and metastatic tumor was insignificant (table 3; $P>0.05$). In the group of germ cell tumors, a large number of TIL (mean range 29.1-39.2) were observed in the stromal tissue of disgerminoma, but a few TIL (range 3.7-11.38) were found in malignant teratoma and endodermal sinus tumor. It was evident, therefore, that heavy infiltration of lymphocytes in disgerminoma raised the mean level of TIL in the whole group of germ cell tumors.

In the present study, 79 of 94 patients were followed up for more than two years. The degree of lymphocyte infiltration in the tumor tissue from these patients was divided into three categories, namely, mild, moderate and marked. The influence of TIL on the survival rate was shown by survival curves (fig. 2). Patients with mild tumor lymphocyte infiltration had a

Table 1. Staging of ovarian malignancies versus degree of TIL

Stage	n	TIL ($\bar{x}\pm s$)	P value versus (4)	P value versus (3)	P value versus (2)
(1) Ⅰ	17	17.12±7.61	<0.01	<0.05	>0.05
(2) Ⅱ	20	16.98±8.33	<0.01	<0.05	
(3) Ⅲ	39	11.47±8.14	>0.05		
(4) Ⅳ	18	8.75±6.88			

Table 2. Grading of ovarian malignancies versus degree of TIL

Grade	n	TIL ($\bar{x}\pm s$)	P value versus(3)	P value versus(2)
(1) Ⅰ	17	16.13±8.25	<0.05	<0.05
(2) Ⅱ	23	8.34±6.28	>0.05	
(3) Ⅲ	7	7.85±5.52		

Table 3. Histologic classification of ovarian malignancies versus degree of TIL

Type	*n*	TIL ($\bar{x} \pm s$)	*P* value versus (4)	versus (3)	versus (2)
(1) epithelial tumor	47	10.73±5.32	>0.05	>0.05	<0.05
(2) germ cell tumor	21	20.92±11.87	<0.01	<0.01	
(3) sex cordstromal tumor	17	9.65±6.14	>0.05		
(4) metastatic tumor	9	7.38±3.59			

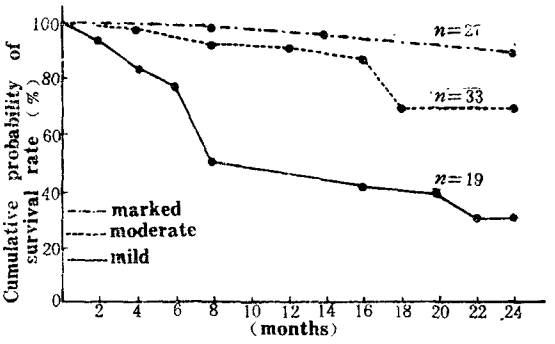

Fig. 2. TIL and survival rate in 79 patients with ovarian malignant tumors.

poor two-year survival rate (31.57%), while the survival rate with moderate and marked tumor lymphocyte infiltration was 69.63% and 88.75%, respectively, the difference being of statistical significance among these three groups ($P<0.05$). These results indicated that patients with pronounced TIL had a high survival rate.

2. **The types of infiltrating cells**

The types of infiltrating cells were identified by frozen section ANAE staining in 15 cases of ovarian malignancies. The major infiltrating cells were lymphocytes which accounted for 66.56% of all the infiltrating cells. Macrophages constituted about 23.66%. Besides, a few polymorphonuclear leukocytes were seen in the stromal tissue. ANAE positive reaction was observed in 58.66% of TIL.

3. **Ultrastructural changes**

In the normal ovary used as control, there were only a small number of normal round lymphocytes with nuclear hyperchromatosis, scanty cytoplasm and organelles (fig. 3). Disintegrated organelles in TIL were seen in only one of the 4 cases of ovarian malignancies, probably due to improper sampling. In the other three specimens, lymphocytes were seen to be in contact with cancer cells. These lymphocytes had a lot of processes on the cell membrane, a big hyperchromatic nucleus with deep notches and plenty of cytoplasmatic organelles including Golgi apparatus and mitochondria. These morphologic observations of the lymphocytes denoted a more active function. Meanwhile, the cancer cells in contact with TIL were seen to show lysis or regressive changes, including cytoplasmic swelling, degeneration and vacuolization of cytoplasmic organelles (fig. 4 and 5).

DISCUSSION

Abnormal relation between tumor cells and the adjacent host cells or tissues is one of the biologic features of tumor. Host-tumor interaction similarly may have direct influence on the biologic behavior of the tumor. Tumor stroma is a component part of tumor on the one hand, and the site of interaction between body effector cells and tumor cells, on the other. Therefore, the number of TIL in the tumor stroma may reflect the host's capability of

inhibiting the proliferation of tumors. In the past, most authors often paid more attention to the tumor itself such as clinical staging, cell grading and histologic typing when they evaluated the prognosis of patients with ovarian malignancies. Recently, however, they began to focus their attention to the important role of TIL and noted that the degree of lymphocyte infiltration in tumor stroma was closely related to the prognosis of the patients.

The results of our study indicated that patients with ovarian malignancies showing high degree of tumor lymphocyte infiltration had a high survival rate, suggesting that the number of TIL in tumor stroma could directly affect the prognosis of the patients. Besides, it was found that a certain relation existed between the degree of lymphocyte infiltration and the clinical staging, cell grading and histological typing. The finding that there were large amounts of TIL in the stroma of disgerminoma could appropriately explain why the patients with this tumor had a relatively high survival rate despite the presence of metastasis and relapses. It is, therefore, believed that TIL may serve as an important indicator in evaluating the prognosis of ovarian malignancies.

The result of frozen section ANAE staining revealed that 58.66% of TIL in the stroma showed a positive ANAE reaction. Such ANAE positive cells are characteristically verified as T lymphocytes, which are believed to be the dominant effector cells in host's local immunity against tumor.

All the above results of morphologic studies could only reflect static situations in the different stages of the pathological process, which might not be taken as good evidence of the interrelationship between TIL and cancer cells. It has even been proposed that the presence of lymphocytes in tumor stroma might be an artefact in that they leaked out from the blood vessels in the course of section preparation. However, electron microscopic examination indicated enhanced functional activity of TIL by a series of findings including increased cell organelles, big and notched nucleus, etc. Meanwhile, the cancer cells seen to be in contact with TIL, were found to have undergone degeneration or even lysis. It is reported that when TIL come into contact with their target cells, the Golgi apparatus and endoplasmic reticulum of the former would soon increase in size and thickness through a series of immunological and biochemical reactions, resulting in the secretion of cytotoxic agents responsible for the destruction of target cells. It is, therefore, justified to reason that infiltration of lymphocytes in the stroma of tumor is an active process of the host to attack tumors cells and inhibit the growth of the tumor.

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