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Immunological Peculiarities of CD-56-Positive Serous Ovarian Adenocarcinoma

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Here we present the results of evaluation of the expression of neural cell adhesion molecules CD56 (NCAM) in serous ovarian adenocarcinoma. The expression was detected in 48.5% cases. Infiltration of tumor stroma and parenchyma with $CD8^+$ и $CD4^+$ lymphocytes was significantly less pronounced in tumors expressing neural cell adhesion molecules; $CD3^+CD4^+CD25^+$ predominate among $CD4^+$ lymphocytes in $CD56^+$ tumors. $CD56^+$ tumors were lower in size (5.2±0.6, 7.9±0.8 and 10.3±1.5 cm in monomorphic, mosaic, and negative phenotypes, respectively (p=0.05) and were characterized by the absence of cystic component (p=0.012), larger disseminations in the peritoneum (4.2±1.1 and 2.7±0.5 cm; p=0.05), and larger volume of the residual tumor (p=0.018) after surgical treatment. NCAM phenotype of the tumor does not correlate with the stage and differentiation degree of serous ovarian adenocarcinoma.

Key Words: *serous ovarian adenocarcinoma; immunohistochemistry; nerve cell adhesion molecules*

CD56 (NCAM, neural cell adhesion molecule) participates in homophilic and heterophilic cell-cell adhesion.

CD56 is presented on subpopulations of NK cell, T killers, cells of brain cortex, follicular epithelium of the thyroid gland, neuromuscular junctions, and neuroendocrine cells. Expression of CD56 was also found in malignant neoplasms of various localizations [3,4,6,7,8,9]. The function of NCAM in human tumors was not studied in detail; experimental studies on animals showed that expression of CD56 in cells of epithelial malignant tumors induces exfoliation of these cells and promotes migration and invasion processes. On the contrary, suppression of NCAM expression reduces adhesion of tumor cells, inhibits their dissemination, and prevents epithelial-mesenchymal transition [5].

Expression of NCAM in serous ovarian adenocarcinoma was first described in 2006 [2]. The presence of this antigen was more typical of less differentiated adenocarcinomas and more disseminated tumor process [2].

Here we studied immunological peculiarities of CD-56-positive serous ovarian adenocarcinoma.

MATERIALS AND METHODS

The study included patients aging 26-77 years (median 50 years; n=33) with first diagnosed serous ovarian adenocarcinoma (IIIC stage in most cases). At stage

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I, surgical treatment was performed (Department of Gynecology, N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences) in 2006-2009.

Immunophenotyping of the tumor was performed on cryostat sections of 33 serous ovarian adenocarcinoma samples by indirect fluorescence reaction. Three frozen tumor sections (4-6 μ) were placed on a degreased slide and fixed in acetone for 10 min for 4°C. Further procedures were performed in a wet chamber at room temperature. The sections were incubated in medium 199 (pH 7.2-7.4) for 10 min. Monoclonal antibodies were applied for 30 min and then the sections were washed in medium199. FITC-labeled $F(ab)_2$-fragments of antiserum against albino mouse globulins were applied for 30 min followed by 10-min washout in medium 199. The cells were preserved in 50% glycerin on physiological saline. The sections were covered with coverslips and antigen-positive cells and structures were characterized. Specific reactions were evaluated under Axioplan microscope at ×500. The results of the reaction were evaluated by a semiquantitative method. Three types of interactions between the antibodies and tumor cells were distinguished [1]: negative (<10% antigen-positive cells), mosaic (10-80% cells) and monomorphic (>80% cells) reaction pattern.

The count of lymphocytes ($CD45^+$, $CD7^+$, $CD3^+$, $CD4^+$, $CD8^+$, $CD56^+$, $CD20^+$) per unit area of 0.34 mm^2 was determined separately for the stroma and parenchyma at ×500. The cells were counted by two independent investigators and the mean value was taken into account.

Specificity of the used monoclonal antibodies was demonstrated (Table 1).

Flow cytofluorometry. The tumor removed during surgery was disintegrated and a cell suspension was prepared on a Medimashin apparatus (BD). The study was performed on a FACScan (BD) using a triple fluorescent label. At least 1000 intratumoral immunocompetent cells ($CD45^+$) were accumulated.

The following antibodies (BD) were used:

1. CD45PE-Cy5/IgG1PE/IgG1FITC,
2. CD45PE-Cy5/CD3PE/IgG1FITC,
3. CD3PE-Cy5/CD8PE/CD4FITC,
4. CD3PE-Cy5/CD25PE/CD4FITC.

Statistical processing of the results included calculation of descriptive statistics, correlation analysis, comparisons of the observed values in groups using nonparametric tests (Mann–Whitney *U* test, Kruskal–Wallis test), and analysis using contingency tables and exact Fisher test.

RESULTS

Immunophenotype of serous ovarian adenocarcinoma was determined in 33 patients (Fig. 1).

Expression of CD56 was monomorphic in 12.1% and mosaic in 36.4%; in 51.5% cases the antigen was not detected on tumor cells. In all cases with CD56 expression, clear-cut and bright fluorescence of tumor cell membranes was observed. Monoclonal antibodies to CD56 also reacted with immunocompetent cells in the tumor tissue.

The type of CD56 expression did not depend on MUC-1, HLA-I, HLA-DR, CD71, CD29 phenotype of serous ovarian adenocarcinoma.

The count of $CD56^+$ intramural lymphocytes was determined 17 patients ($CD56^-$ tumors). The content of $CD56^+$-cells in tumor parenchyma varied from 0 to 50 (mean 8.09±3.20 cells) and in the stroma from 0 to 50 (mean 6.70±3.08 cell). The number of $CD56^+$ tumor-penetrating lymphocytes correlated with the number of $CD7^+$ (R=0.51; p=0.03), $CD8^+$ (R=0.62; p=0.008), and $CD4^+$ (R=0.58; p=0.01) lymphocytes. The number of $CD56^+$ stromal cells correlated with the number of $CD7^+$ (R=0.51; p=0.03) and $CD4^+$ (R=0.51; p=0.04) lymphocytes. The degree of infiltration with $CD56^+$ tumor-penetrating and stromal lymphocytes did not depend on immunophenotype of serous ovarian adenocarcinoma.

TABLE 1. Specificity of Applied Monoclonal Antibodies

Antigen/CD/MKA	Molecule
I. Antigens of tumor cells	
KL-1/KL-1	Cytoketarins
MUC-1/CD227/ICO-25	Mucin-1
HLA-I/ICO-53	Class I histocompatibility molecules
HLA-DR/ICO-I	Class II histocompatibility molecules
VLA-β/CD29/K-20	Integrin β-chain
CD71/ICO-92	Transferrin receptor
N-CAM/CD56/My-31	Neural cell adhesion molecule
II. Antigens of immunocompetent cells	
CD45/ICO-58	Leukocytes
CD7/ICO-87	T cells; natural killer subpopulation
CD3/ICO-90	Mature T cells
CD8/ICO31	Cytotoxic T cells; natural killer subpopulation
CD4/ICO-86	T-helpers, regulatory T cells
CD56 (N-CAM)/My31	Natural killers
CD20/L-26	B cells

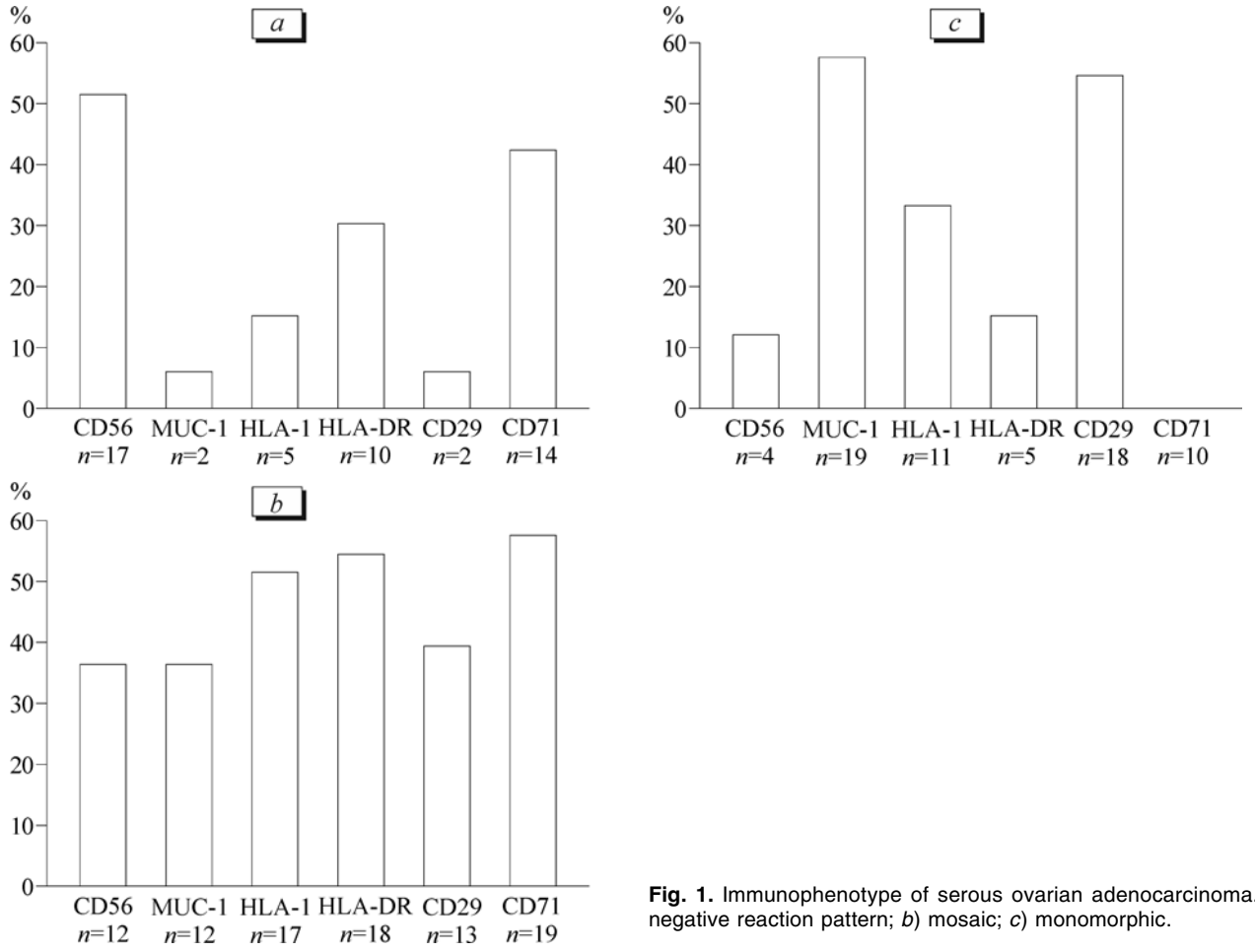

Fig. 1. Immunophenotype of serous ovarian adenocarcinoma. *a*) negative reaction pattern; *b*) mosaic; *c*) monomorphic.

$CD56^+$ tumors were characterized by less pronounced infiltration with tumor-penetrating and stromal lymphocytes of all studied populations (Table 2). Significant differences were revealed for $CD8^+$- и $CD4^+$ lymphocytes of tumor stroma and parenchyma.

According to additional flow cytofluorometry analysis (18 patients), there were no significant differences between $CD56^-$ и $CD56^+$ variants of serous ovarian adenocarcinoma in the percentage of $CD3^+$ intratumoral cells and their subpopulations (CD4 and

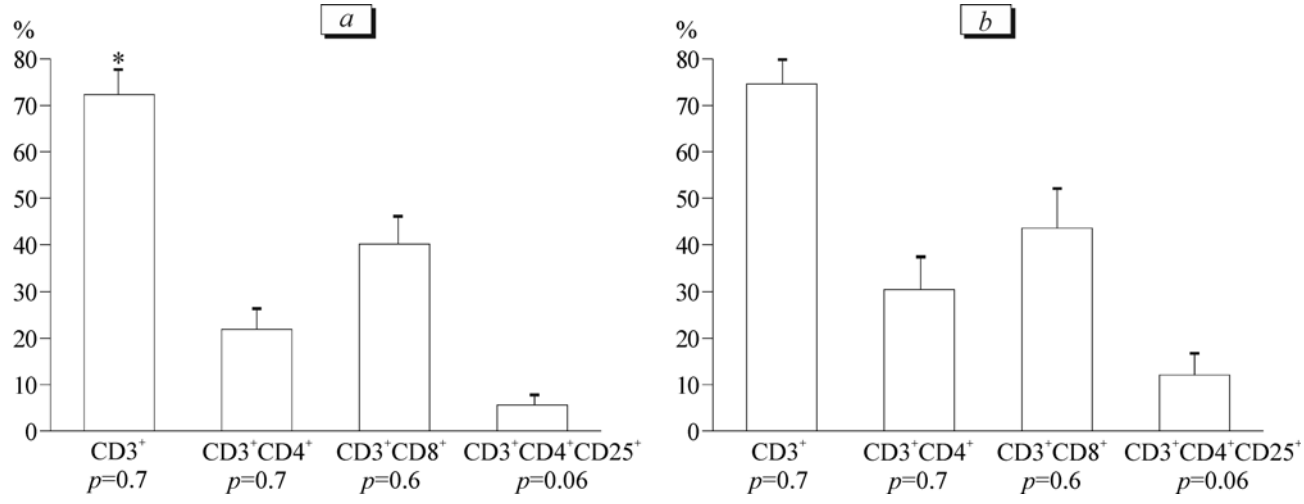

Fig. 2. Correlation between the expression of CD56 by cells of serous ovarian adenocarcinoma and intratumoral infiltration with subpopulations of mature T cells ($M \pm m$). *a*) negative CD56 reaction pattern; *b*) positive. *Among intratumoral lymphocytes $CD45^+$.

TABLE 2. Expression of CD56 by Serous Ovarian Adenocarcinoma Cells and Infiltration with Intratumoral Lymphocytes (*M*±*m*)

Marker	CD56 (NCAM) reaction pattern		*p*
	negative	mosaic and monomorphic	
$CD45^+$ TPL	67.7±20.2	55.9±16.1	0.2
$CD45^+$ stroma	69.7±22.1	85.1±24.3	0.6
$CD7^+$ TPL	18.2±7.2	9.1±2.5	0.2
$CD7^+$ stroma	12.0±2.8	10.4±3.4	0.6
$CD3^+$ TPL	12.2±3.9	7.5±2.2	0.1
$CD3^+$ stroma	24.8±8.9	13.7±3.8	0.08
$CD8^+$ TPL	16.0±5.3	8.7±2.8	0.01
$CD8^+$ stroma	18.9±6.5	8.5±2.5	0.002
$CD4^+$ TPL	10.1±4.9	3.9±1.4	0.03
$CD4^+$ stroma	14.1±6.0	6.5±2.1	0.02

Note. TPL: tumor-penetrating lymphocytes

CD8, Fig. 2). $CD56^+$ tumors are characterized by significantly higher content of intratumoral lymphocytes with a phenotype of T-regulatory (suppressor) cells, $CD4^+CD25^+CD3^+$ (Fig. 2).

Thus, $CD56^+$ tumors are characterized by significantly less pronounced reaction of intratumoral lymphocytes, in particular T-killers ($CD8^+$) penetrating into the tumor zones, and increased content of T-regulatory cells suppressing the antitumor T-cell immune response. All these features suggest that antitumor immune response can be less pronounced or suppressed in patients with $CD56^+$ tumors.

Of particular interest is the relationship between CD56 expression and clinical peculiarities of the tumor process. $CD56^+$ serous ovarian adenocarcinoma is characterized by solid structure (cyctic component was absent in all cases) and smaller size of the primary tumor. At the same time, larger disseminations in the peritoneum and larger residual tumor after surgical treatment were noted (Table 3). These peculiarities can be explained by the fact that expression of CD56 molecules in serous adenocarcinoma promotes adhesion of tumor cells to each other (homophilic adhesion) and less affects their adhesion to the mesothelium.

The expression of this marker was not related to patient's age, menstrual function, previous gynecological diseases, stage of serous adenocarcinoma (p=0.5), degree of tumor differentiation (p=0.16), initial serum level of CA 125 (p=0.9), metastatic involvement of the greater omentum (p=0.6), and signs of peritoneal carcinomatosis (p=0.7).

Thus, characteristics of growth and dissemination of $CD56^+$ serous ovarian adenocarcinoma can be explained by peculiarities of intratumoral compartment of the antitumor T-cell immunity.

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TABLE 3. Clinical Peculiarities of CD56 (NCAM)-Positive Tumors (*M*±*m*)

Macrostructure of the tumor	CD56 (NCAM) expression pattern			*p*
	monomorphic	mosaic	negative	
Cystic-solid/solid, %	0/100	83.3/16.7	58.8/41.2	0.012
Tumor size, cm	5.2±0.6	7.9±0.8	10.3±1.5	0.05
Size of peritoneal disseminations, cm	4.2±1.1	2.7±0.5	0.05	
Size of residual tumor, cm	3.4±0.6	6.8±1.3	6.0±0.6	0.018

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