Gains and losses of CD8, CD20 and CD56 expression in tumor stroma-infiltrating lymphocytes compared with tumor-associated lymphocytes from ascitic fluid and lymphocytes from tumor draining lymph nodes in serous papillary ovarian carcinoma patients

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Summary

Serous papillary ovarian cancer (SPC) is a highly aggressive tumor. About two-thirds of women have advanced disease at the time of diagnosis. Aithough many women with disseminated disease respond at first to combinations of surgery and chemotherapy, nearly 90% of tumors recur and women die of disease. Update progress in our knowledge of tumor-associated antigens and insight into mechanisms involved in immune-mediated recognition of these antigens, have provided a strong starting point for using the immune system as a model for novel therapy. In this study we determined the immunological profile of tumor-infiltrating lymphocytes (TILs), tumor-associated lymphocytes (TALs) in ascitic fluids, and lymphocytes from tumor draining regional lymph nodes (LNs) in SPC patients by CD20 (L26), CD8, and CD56 immunostaining. We examined 14 cases of TILs, 15 cases of TALs and 19 cases of LNs. TILs were infiltrating tumor stroma. No significant difference was detected in TILs, TALs and LNs in the expression of the B-cell marker CD20. In contrast, CD8 (T-cytotoxic) and CD56 (natural killer cell, NK) markers were dominant in LNs and TALs, but not in TILs. We conclude that SPC tumor lymphocytic infiltrate demonstrates a deplete T cytotoxic (CD8+) and NK cell (CD56+) immunophenotypic profile. This might in part explain the poor clinical outcome of the disease.

Key words: Serous papillary ovarian carcinoma; Ascitic fluid; Tumor infiltrating lymphocytes; Tumor associated lymphocytes; Tumor draining lymph nodes; CD8, CD20, CD56.

Introduction

Ovarian epithelial cancer (adenocarcinoma) is responsible for the largest number of deaths from malignancies of the female genital tract and is the fifth leading cause of cancer death in women [1]. Most ovarian adenocarcinomas are of the serous histological type (Figure 1) [1]. Clinically, about two-thirds of serous neoplasms of the ovary present de novo as advanced-stages tumors, reflecting their propensity for intra-abdominal/peritoneal spread (Figure 2) [1, 2]. These tumors arise from tranformed cells of the coelomic surface epithelium of mullerian origin which accounts for their ontogenetic and phenotypic kinship, histological overlap, and sometimes coexistence with carcinomas of the endometrium and endocervix [3-5]. Occasionally, identical serous neoplasms may arise from the so-called secondary mullerian system [6] involving the pelvic and lower abdominal mesothelium. These extraovarian serous adenocarcinomas, or papillary tumors of the peritoneum, are very closely related to their ovarian counterparts and are different, both in terms of phenotype and clinical behavior, from the mesotheliomas of the peritoneum [5, 7, 8]. Ovarian carcinomas, like carcinomas of the breast and endometrium, are steroid hormone-dependent epithelial neoplasms. One of the unifying features of female genital cancer is the presence of steroid receptors in tumor cells, including estrogen, progesterone, and androgen receptors [9].

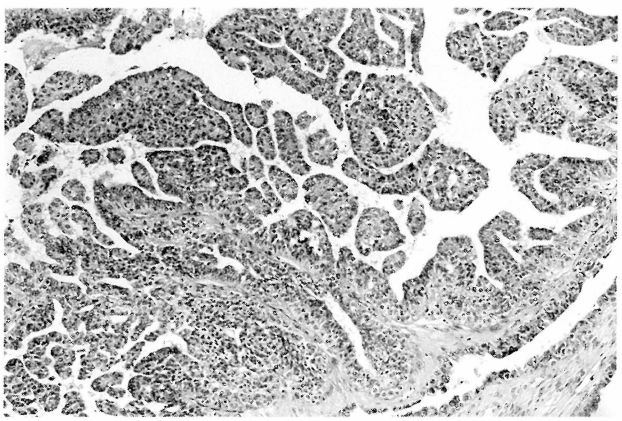

Figure 1. — Papillary serous adenocarcinoma of ovary with loss of orientation and piling up of atypical epithelium. A dense lymphocytic infiltrate is observed in the tumor stroma. HEx100.

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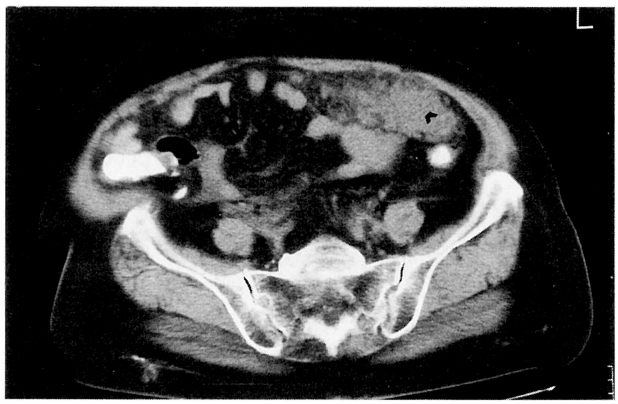

Figure 2. — CT scan: Peritoneal spread of serous papillary ovarian carcinoma (arrow head).

The CD20 antigen is a non-glycosylated phosphoprotein of approximately 33KD which is expressed on normal and malignant human B cells and is thought to act as a receptor during B cell activation and differentiation. NCL-CD20-L26 is an established and well characterised clone for use in the detection of CD20 antigen. NCL-CD20-L26, NCL-CD20-7D1, NCL-CD20-MJ1 and NCL-CD20p may be used for the detection of CD20 antigen on normal B cells from peripheral blood, lymph nodes, spleen, tonsils, bone marrow and in the classification of acute leukemias and chronic lymphocytic leukemias.

The CD8 molecule is composed of two chains and has a molecular weight of 32KD, It is found on a T-cell subset of normal cytotoxic/suppressor cells which make up approximately 20 to 35% of human peripheral blood lymphocytes. The CD8 antigen is also detected on natural killer (NK) cells, 80% of thymocytes, on a subpopulation of 30% of peripheral blood null cells and 15 to 30% of bone marrow cells. NCL-CD8-295, developed to produce superior staining, or NCL-CD8-4B11 may be used in conjunction with other T-cell markers to type human leukaemias and lymphomas of T-cell origin. NCL-CD8-295, NCL-CD8-4B11 and NCL-CD8 may be used in assessing the nature of lymphoid cell infiltrates present in biopsy specimens and to distinguish between reactive and neoplastic T cells.

The neural cell adhesion molecules are a family of closely-related cell surface glycoproteins thought to play a role in embryogenesis, development and contact-mediated interactions between neural cells. The CD56 antigen (NCAM) consists of four major isoforms generated by differential splicing of the RNA transcript from a single gene located on chromosome 5. The CD56 antigen is expressed on neurons, astrocytes, Schwann cells, NK cells and a subset of activated T lymphocytes. NCL-CD56-1B6 and NCL-CD56 may prove useful in the determination of neuroectodermal tumor origin and especially in the delineation of residual neuroblastoma.

In our study we investigated the immunophenotypic profile of tumor stroma infiltrating lymphocytes (TILs), tumor-associated lymphocytes (TALs) from ascitic fluid and lymphocytes from regional lymph nodes (LNs) draining tumor cells in serous papillary ovarian carcinoma.

Materials and methods

We studied 14 cases of tumor infiltrating lymphocytes (TILs), 15 cases of tumor-associated lymphocytes (TALs) from the ascitic fluid using cell block preparations, and 19 cases of lymphocytes from regional lymph nodes (LNs) collected from patients harboring advanced serous papillary ovarian carcinoma.

Tissue samples were processed for paraffin-section immunophenotyping and stained using the monoclonal antibodies CD8, CD20, and CD56 (NOVOCASTRA).

The immunostained sections were examined with a x 40 objective and the distribution of CD8, CD20, and CD56 within the cell was recorded. Every stained cell was scored as positive regardless of staining intensity. To count the number of cells with CD8, CD20, and CD56 stainings, a 10 x 10 square calibrated grid was inserted into the eyepiece of an Olympus binocular microscope.

Five-to-ten fields were examined for each section, ant at least 1,000 cells were scored, depending on cellularity. The percentage of positive cells was recorded as the CD8, CD20, and CD56 indices.

$$\text{CD8 index} = \frac{\text{no. of positive cells}}{\text{no. of total (positive+negative cells)}}$$

$$\text{CD20 index} = \frac{\text{no. of positive cells}}{\text{no. of total (positive+negative cells)}}$$

$$\text{CD56 index} = \frac{\text{no. of positive cells}}{\text{no. of total (positive+negative cells)}}$$

The CD8, CD20, and CD56 indices ranged from 0-100%, with a mean of 18%. The mean index was evaluated in three ranges: low index (under 18%), grade I; moderate index (from18 to 50%), grade II; and high index (from 51 to 100%), grade III.

Results

The sections were examined independently by two observers, and positive cellular staining for CD8, CD20, and CD56 antibodies were manifested as fine yellow cytoplasmic granularity and/or surface membrane expression (Figures 3, 4).

CD20 was expressed in all 14 cases of TILs (100%), in all 15 cases of TALs (100%), and in 18 of 19 cases of LNs (94.73%).

CD56 was expressed in nine of 14 cases of TILs (64.28%), in 11 of 15 cases of TALs (73.33%), and in 17 of 19 cases of LNs (89.47%).

CD8 was expressed in eight of 14 cases of TILs (57.14%), in ten of 15 cases of TALs (66.66%), and in 18 of 19 cases of LNs (94.73%).

Of 14 CD20 positive TILs five were scored as grade I, seven as grade II, and two as grade III.

Of 15 CD20 positive TALs five were scored as grade I, seven as grade II, and three as grade III.

Of 18 CD20 positive LNs three were scored as grade I, eight as grade II and seven as grade III.

Of nine CD56 positive TILs seven were scored as grade I, and two as grade II. Of 11 CD56 positive TALs eight were scored as grade I, two as grade II, and one as grade

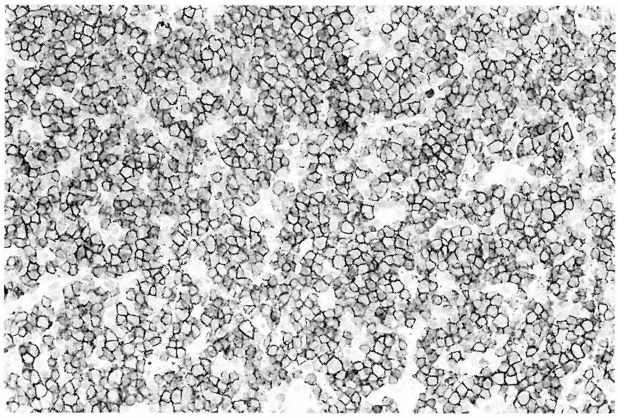

Figure 3. — Papillary serous adenocarcinoma stroma stained for the CD56 antigen, showing a dense pattern of involvement. Immunostaining x100.

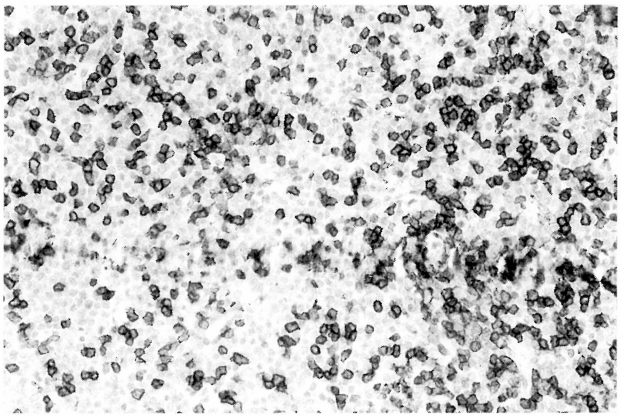

Figure 4. — Papillary serous adenocarcinoma stroma for the CD8 antigen showing a loose pattern of involvement. Immunostaining x100.

III. Of 17 CD56 positive LNs five were scored as grade I, seven as grade II and five as grade III.

Of eight CD8 positive TILs five were scored as grade I, two as grade II, and one as grade III. Of ten CD8 positive TALs six were scored as grade I, and four as grade II. Of 18 CD8 positive LNs seven were scored as grade I, ten as grade II, and one as grade III.

Discussion

Natural killer cells (CD56+, CD57+) constitute a pool of defensive elements which appear to have functional similarities to cytotoxic T cells, although they lack some typical lymphocyte features. They normally form only a small percentage of all lymphocyte-like cells and are included technically in the "large granular lymphocyte" category. Natural killer cells, when mature, have a mildly basophilic cytoplasm and a partially euchromatic nucleus. Ultrastructurally, the cytoplasm contains ribosomes, granular endoplasmic reticulum and dense, membrane-bound vesicles 200–500 nm in diameter with crystalline cores. These contain some hydrolases, but the major active component is a protein, cytolysin, capable of inserting holes in the plasma membranes of other cells, so causing their death. Natural killer cells are activated to attach themselves to and kill target cells of various kinds by a number of factors, including IL-2 from T cells. They represent a relatively non-specific means of attacking virus-infected cells, protozoa and other pathogenic cells.

When monoclonal antibodies were raised against cell surface antigens of human lymphocytes, it was found that different classes of T lymphocytes could be grouped together according to the characteristic range of monoclonal antibodies they bound. When these cell surface molecules were finally identified by the determinants recognized on them by monoclonal antibodies, they were given an international cluster determinant (CD) number. Nowadays, CD numbers define a very large number of cellular molecules which have been cloned and sequenced and whose biological functions are wholly or partially characterized [10]. All "true" T cells express the CD3 molecular complex, which is responsible for signal transmission, mediated via the T-cell receptor (TCR) they also express either CD4 or CD8. Those which bear the CD4 molecule (CD4+) include helper-inducer cells important in triggering antibody production from B lymphocytes, cytotoxic T cells, and T cells involved in delayed hypersensitivity reactions. Those bearing the CD8 molecule (CD8+) comprise cytotoxic T cells and others with suppressor functions on other cell types. Natural killer cells are CD3 positive, but do not normally express CD4 or CD8 markers. This scheme of classification is by no means absolute in terms of relating T lymphocyte phenotypes to a particular function. At present the CD4 population has been subdivided into three subgroups (Th0, Th1, Th2) based on the mix of cytokines released by these T cells following antigen stimulation. These CD molecular complexes are believed to act cooperatively with T-cell receptors, to mediate stimulus transduction and activation of a number of cellular functions. Both CD4 and CD8 molecules can be regarded as functioning as co-receptors to the TCR in the recognition of antigen, and are involved in the signal transduction from the cell surface to the nucleus to initiate 'helper' or other related activities, or in the case of CD8 to initiate cytotoxic activity, or 'suppressor' functions, etc. Although the plethora of activities carried out by lymphocytes seems highly complex, it is to be expected that the potent and wide-ranging defensive mechanisms of the body should be subject to multiple checks, controls and regulations. As yet, relatively little is understood about the manner in which the various parts of the whole system of cellular and chemical defences are integrated, but it is increasingly clear they must be viewed as a single system of great efficiency and elegance. When, however, such integration breaks down, the effects may be far reaching as, for example, in the wide variety of autoimmune diseases that occur in man, and in neoplasia of the immune system, such as myeloma.

In our series, consistent with previous reporting [11-24], we found that specific CD8+ cytotoxic T-lymphocy-

tes and CD56+ natural killer cells which infiltrate the stroma of the serous papillary ovarian carcinoma and kill autologous neoplastic cells are diminished in women harboring this tumor resulting in a poor clinical outcome.

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