ONCOLOGY

Interactions Between Natural Autoantibodies to Tumor-Associated Human Ovarian Cancer Antigen and Murine Ovarian Cancer Cells CaO-1

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The interactions between tumor cells and autoantibodies to tumor-associated human ovarian cancer antigen isolated from the plasma of a non-cancer patient are demonstrated using an original model of pseudomucinous murine ovarian carcinoma CaO-1. The use of natural autoantibodies for active immunotherapy of tumors may be more effective and safe than murine monoclonal antibodies to CA 125, because there will be no reaction to a foreign protein.

Key Words: *natural autoantibodies; human ovarian cancer; murine ovarian cancer*

Study of tumor-associated antigens (TAA) detected in cancer patients and their role in the diagnosis and treatment of malignant tumors is an important trend of cancer research [3]. TAA are high-molecular-weight glycoproteins with extremely conservative structure, which may be indicative of their important biological role. For example, the peptide sequences of human and chimpanzee MUC 1 antigen differ by only 1% [2].

There are no published data on the homology of glycoproteins expressed in human and murine ovarian cancer. In the present study we attempted to detect common antigenic determinants for glycoproteins expressed in human ovarian cancer and experimental mouse tumors of the same origin. This study was prompted by the detection of high titers of autoantibodies (autoAB) to human ovarian cancer TAA in the plasma of a non-cancer patient. Natural autoAB purified by affinity chromatography were used for examining the TAA of homologous murine tumors.

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The data can be used for studying natural autoAB and TAA to glycoprotein CA 125 expressed in patients with ovarian cancer and for detecting TAA homology in humans and mice with histologically similar tumors.

MATERIALS AND METHODS

The study was carried out on an original model of murine mixed pseudomucinous ovarian carcinoma CaO-1 obtained and maintained in CBA mice. Similar tumors often occur in humans. This cell strain (frozen at the second generation) is highly sensitive to cytostatics routinely used in human ovarian cancer. We assume that the antigenic characteristics of this tumor remained unchanged. Relatively rapid growth of the tumor also is an important factor.

Female CBA mice aged 2-3 months were used. CaO-1 ovarian cancer cells were transplanted subcutaneously in a dose of 10^6 cells in 0.3 ml medium 199. The cells were isolated from the tumor node on day 21 after tumor transplantation.

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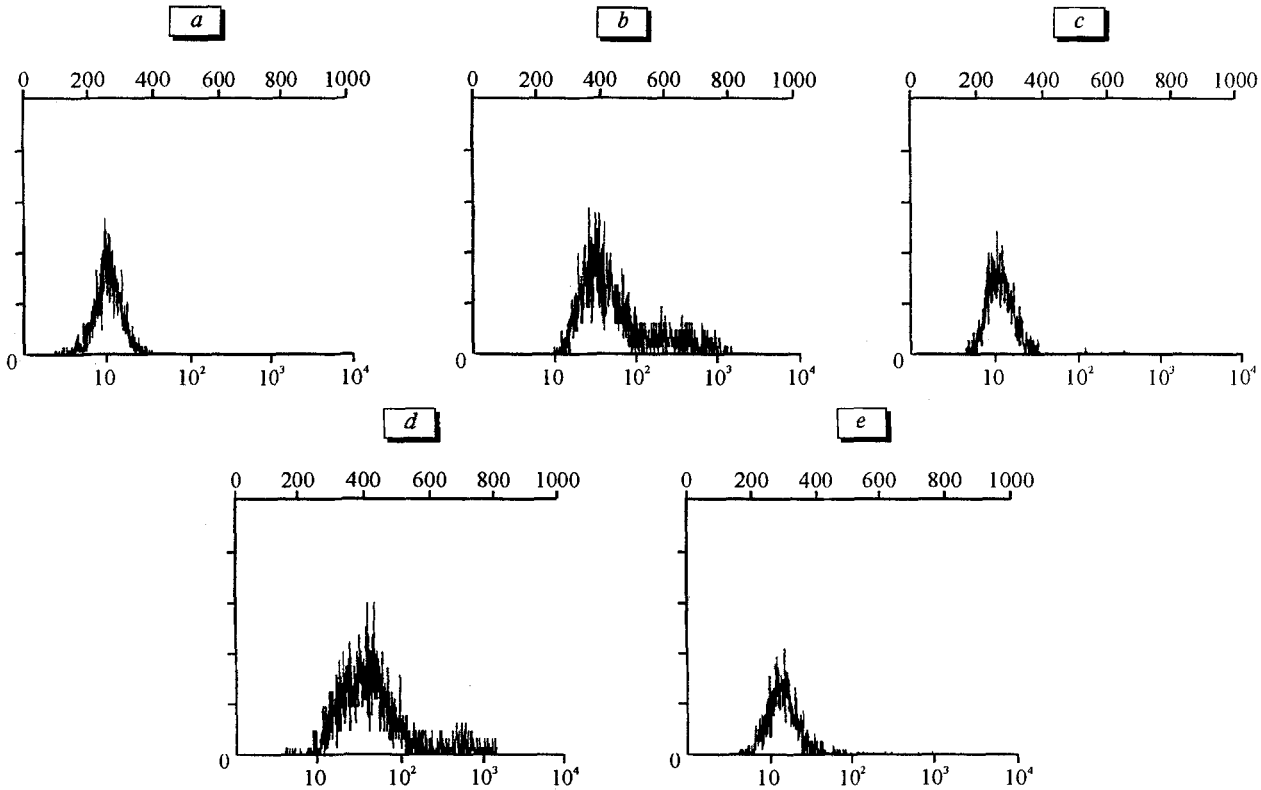

Fig. 1. Fluorescence of murine ovarian cancer cells CaO-1 in the control (*a*) and after incubation with FITC-labeled (*b*) and unlabeled (*c*) autoantibodies to tumor-associated antigen of human ovarian cancer. *d*) staining by indirect fluorescence (using second antibodies); *e*) staining with second antibodies. Abscissa: cell fluorescence, arb. U; ordinate: number of fluorescent cells, arb. U; upper scale: cell size.

Highly purified CA 125 antigen was isolated from ascitic fluid of an ovarian cancer patient and immobilized on Sepharose 4B in a concentration of 5×10^5 U/ml gel. The binding capacity of the immunosorbent in the model experiments with murine monoclonal anti-CA 125 antibodies (clone X 75) was about 2 mg AB/ml gel.

Natural human autoAB to ovarian cancer antigens were isolated from pretreated plasma with a high titer of these AB. The plasma was diluted with 0.5 M NaCl, transferred to immunosorbent, and bound AB were then eluted with 0.1 M acetate buffer (pH 2.5). The yield of AB was about 200 μg/ml plasma. The ability of eluted AB to bind CA 125 antigen was verified by enzyme immunoassay.

The AB eluted with acid buffer were labeled with FITC [1].

Murine ovarian cancer cells CaO-1 were stained by incubation with primary FITC-labeled AB or by using second AB to human immunoglobulins.

Fluorescence of stained cells was measured on a Facscan cytofluorometer (Becton-Dickinson).

RESULTS

Baseline fluorescence of nonstained CaO-1 cells was used to establish a zero signal. The fluorescence of tumor cells after incubation with primary FITC-labeled AB is shown on Fig. 1, *b*. A shift of the peak to the right corresponds to accumulation of fluorescent cells, while the "shoulder" of the fluorescence curve probably reflects high variability in cell size (Fig. 2). The same curves were obtained using the method of indirect fluorescence (staining with second AB) (Fig. 1, *d*). Tumor cells treated with nonlabeled primary AB

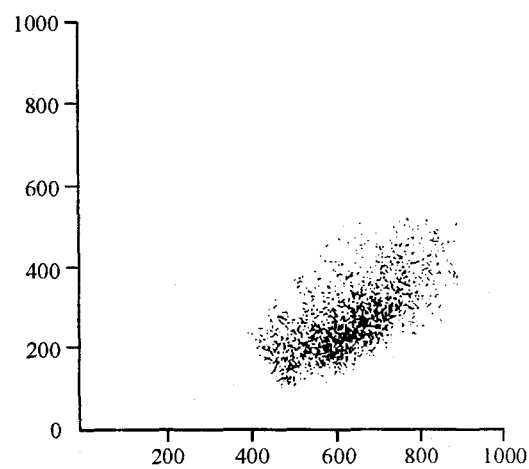

Fig. 2. Murine ovarian cancer cells CaO-1. Abscissa and ordinate: cell size.

TABLE 1. Fluorescence of CaO-1 Murine Ovarian Cancer Cells after Incubation with Natural AutoAB

Groups	% of fluorescent cells
Control	0
FITC-labeled autoAB	66
AutoAB	1
AutoAB+second AB	63
Second AB	6

(Fig. 1, *c*) or only second AB (Fig. 1, *e*) did not fluoresce. Quantitatively, the levels of fluorescence of specifically stained cells virtually coincided (Table 1).

Two heretofore unknown phenomena were observed in this study: 1) the presence of autoAB to ovarian cancer TAA in the serum of a nononcological patient and 2) common antigenic determinants in TAA expressed by human and mouse tumors of similar genesis. Despite the rigorous conditions of isolation, the resultant autoAB retained high affinity to both human and murine TAA. Use of natural autoAB for active immunotherapy of tumors with consideration for their high affinity may be more effective and safe than the use of murine monoclonal antiCA 125 AB because of the absence of immunological reactions to a foreign protein. It should be noted that even a single injection of Ovarex to patients with ovarian cancer leads to the appearance of human anti-mouse antibodies in the serum.

On the other hand, the role of AB to ovarian cancer glycoproteins in AB-dependent cell cytotoxic reactions is unknown, and the fact of their interaction with murine tumor cells provides the basis for experimental model in these studies.

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