# ONCOLOGY

# Evaluation of Immunotherapy Efficiency in Mouse CaO-1 Ovarian Carcinoma Treated by Vaccines Based on Dendritic Cells

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Injection of dendritic cells, pulsated by tumor lysate or mucin, containing CA 125 antigen, led to a more than 50% inhibition of tumor growth in female CBA mice with transplanted mouse pseudomucinous CaO-1 ovarian carcinoma in comparison with the control. Tumor-associated CA 125 antigen can be used for obtaining dendritic cell vaccines against ovarian malignant tumors. This trend will extend the potentialities of application of antitumor vaccines based on dendritic cells, as clinical use of this technology is limited by the need in patient's tumor material. Mucin, containing Ca 125 antigen, can be isolated from patient's serum or obtained by gene engineering technologies as a recombinant peptide.

**Key Words:** *dendritic cell vaccines; CaO-1 mouse ovarian carcinoma; tumor lysate; CA 125 antigen*

Progress in understanding of the functional interactions between tumor cells and host immune system, particularly stimulation and regulation of T-cellular response, gave a new impetus to studies of antitumor vaccines. The critical event for induction of antitumor response is capture, processing, and presentation of tumor antigens by T-lymphocytes, professional antigen-presenting cells (APC). Dendritic cells (DC) are potent APC with a unique capacity to stimulate the primary immune response. Experimental studies have shown that DC vaccines activate specific cytostatic T-cells (CTL) and cause tumor regression. These data and development of methods for the production of high amounts of DC *ex vivo* led to the use of DC for presentation of tumor antigens to the immune system effectors for induction or stimulation of inadequate response. The capacity of DC, pulsated by tumor antigens, to induce CTL activation, was documented in several clinical studies in patients with different tumors, including B-cell lymphoma, myeloma, melanoma, prostatic cancer, *etc*. Approaches to creation of DC vaccines against ovarian malignant tumors are developed in recent years [3-6]. Comparison of DC pulsated by RNA and tumor lysates showed their equal efficiency under experimental conditions. A promising trend in creation of DC-based vaccines is use of tumor-associated antigens [3].

We studied the effects of DC vaccines, pulsated by tumor lysate or mucin containing CA 125 antigen, on the time course of mouse pseudomucinois CaO-1 ovarian carcinoma growth. This strain is

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selected as the experimental model because it has common antigenic determinants with CA 125 antigen, expressed in the majority of patients with ovarian malignant tumors [1,2]. In addition, tumors of similar structure are rather incident in the patients.

## MATERIALS AND METHODS

The study was carried out on female CBA mice (2-3 months) from Stolbovaya Breeding Center (Moscow Region). Mouse CaO-1 ovarian carcinoma was transplanted subcutaneously ($10^6$ cells in 0.3 ml medium 199).

Tumor cell lysate was prepared by three freezing (-70°C) and defrosting cycles of the cells isolated from the tumor node on day 21 after transplantation.

Mucin, containing CA 125, was obtained as described previously [1,2].

Dendritic cells were derived from the bone marrow cells of 30 CBA females. The bone marrow was homogenized in RPMI-1640 (Sigma), precipitated 3 times by centrifugation (250*g* during 5 min), and inoculated in enriched culture medium ($10^6$ cell/ml RPMI-1640 with 100 μg/ml gentamicin sulfate and 10% heat-inactivated FCS), containing recombinant granulocytic macrophageal CSF and IL-4 (Biosource), 10 ng/ml each. On day 6 the medium was replaced and polycomponent VP-4 vaccine (50 μg/ml) was added for induction of DC maturing. Mature DC were incubated during 24 h with tumor lysate or mucin, containing CA 125.

Dendritic cell vaccines, pulsated by tumor lysate or mucin containing CA 125, were injected subcutaneously ($10^6$) cells twice at 2-week interval. Tumor cells (CaO-1) were transplanted 2 weeks after revaccination. Controls received 2 injections of nonpulsated DC, tumor lysate, or saline at 2-week intervals. The treatment efficiency was evaluated by the time course of tumor growth in the control and experimental groups on days 10 and 20 after transplantation. Each group consisted of 20 animals. The data were statistically processed by Fisher—Student's method. The differences were considered significant at $p<0.05$.

## RESULTS

Studies of the immunophenotype of DC, generated from mouse bone marrow, showed virtually no $CD34^+$ precursor cells, which served as a source of DC. High level of CD80 and CD86 costimulatory molecules, expression of class II MHC, and the morphology (polygonal cells with multiple cytoplasmatic processes) indicated that this population could be classified as mature DC (Table 1, Fig. 1).

**TABLE 1.** Immunophenotype of DC Generated from CBA Mouse Bone Marrow

| Differentiation antigen | Level of expression *vs.* total count of DC, % |
|---|---|
| MHC II | 50.4±18.0 |
| CD40 | 83.7±21.0 |
| CD80 | 34.7±9.0 |
| CD34 | 3.1±1.0 |
| CD86 | 43.6±8.0 |

Injection of DC vaccine, pulsated by tumor lysate, to mice with transplanted CaO-1 caused a more than 50% inhibition of tumor growth. Injection of nonpulsated DC and tumor lysate caused just negligible shifts. Vaccination of mice by DC charged by mucin containing CA 125 was no less effective that vaccination by DC pulsated by tumor lysate. Injection of mice with mucin containing CA 125 antigen led to a slight (up to 35%), though significant inhibition of tumor growth (Table 2).

Hence, use of DC as APC stimulates the immunogenic activity of tumor antigens. Equal efficiency of DC vaccines, charged by mucin containing CA 125, and DC vaccines, pulsated by cell lysate, indicates that tumor-associated CA 125 antigen can be used for obtaining DC vaccines for protection from ovarian malignant tumors. We think that this trend of research will extend the potentialities of using DC-based antitumor vaccines, as the clinical application of this technology is limited

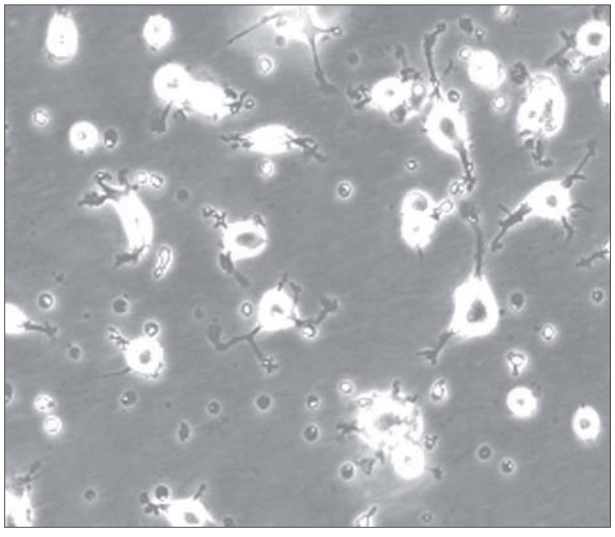

**Fig. 1.** Mature DC generated from CBA mouse bone marrow precursor cells. Phase contrast microscopy of culture suspension, ×400.

**TABLE 2.** Effects of DC Vaccines Pulsated by Tumor Lysate or Mucin Containing CA 125 Antigen on the Time Course of Mouse CaO-1 Ovarian Carcinoma Growth

| Group | Mean tumor volume after transplantation | | Tumor growth inhibition after transplantation* | |
|---|---|---|---|---|
| | day 10 | day 20 | day 10 | day 20 |
| Control | 3232.0±117.9 | 7430.0±1232.8 | | |
| DC | 2443.0±312.3 | 5800.0±329.1 | 25 | 22 |
| Tumor lysate | 2701.0±212.3 | 6087.0±429.1 | 17 | 19 |
| DC+tumor lysate | 1687.0±219.2 | 3739.0±213.2 | 52+ | 50+ |
| CA 125 | 2286.0±232.4 | 4901.0±213.2 | 30 | 35+ |
| DC+CA 125 | 1487.0±318.1 | 3201.0±227.4 | 54+ | 57+ |

**Note.** *Percent of control. +$p<0.01$ compared to control.

by the need in tumor material from the patient. Mucin, containing CA 125 antigen, can be isolated from patient's serum or obtained by gene engineering technologies as a recombinant peptide.

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