IMMUNOMORPHOLOGIC STUDIES OF AN ANTIGEN ASSOCIATED WITH HUMAN CERVICAL SQUAMOUS CELL CARCINOMA

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Carcinoma of the cervix uteri (CCU) is one of the commonest malignant neoplasms of the female reproductive organs. Cytologic screening, and also adequate and timely treatment of predisposing causes, precancerous states, and preinvasive carcinoma contribute to a decrease in the incidence of CCU and mortality from it. Meanwhile, one important problem connected with the development of new approaches to the early diagnosis, methods of monitoring the efficacy of treatment, and discovery of subclinical recurrences and metastases, still remains unsolved. One way of improving the sensitivity of diagnostic and prognostic methods in oncogynecology in recent times has been the search for and use of immunologic tumor-associated markers [4, 5]. This paper describes the results of an immunohistochemical and immunocytochemical study of pretumor diseases and tumors of the cervix uteri (CU), based on the discovery of a previously identified antigen, associated with squamous-cell CCU (ASCCU) [1] in test material, with the aim of determining its importance as an immunologic marker for this tumor.

EXPERIMENTAL METHOD

Immunocytohistochemical tests were carried out on preparations of tissue sections and films obtained from patients with pathology of CU and also from clinically healthy women, using the indirect immunofluorescence test (IFT) [3] and the indirect immunoperoxidase method (IPM) [7]. During the immunohistochemical investigation biopsy material obtained from 52 patients with CCU and dysplasias, and also cadaveric material from 10 women without pathology of CU, was used. Pieces of tissue were fixed in a mixture of acetone, formalin, and 0.03 M phosphate buffer, pH 6.2 (9:5:6) and embedded in histoplast. Serial sections 4 μ thick were dewaxed by the standard method. During IFT the sections were treated with antibodies to ASCCU in dilutions of 1:8 and 1:16 for 30 min at 37°C, and after washing in buffered physiological saline, pH 7.4 (BPS), luminescent serum against rabbit Ig (1:16) (N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) was applied as secondary antibodies. The sections were then mounted in 50% glycerol solution, pH 7.2, and examined in the luminescence microscope. To record the nonspecific background, before application of the primary antibodies the sections were treated with a 1% solution of bovine serum albumin for 15 min. Endogenous peroxidase was inhibited by 0.03% H_2O_2 solution in methyl alcohol. The above-mentioned antibodies were used as primary antibodies, and antibodies against rabbit Ig labeled with peroxidase (1:32) (N. F. Gamaleya Institute) as secondary. The reaction product was developed with 0.05% solution of 3,3'-diaminobenzidine in phosphate buffer, pH 7.4, with the addition of 0.35% H_2O_2. After the reaction had ceased, the sections were counterstained with hematoxylin. As the control, instead of primary antibodies, either BPS, pH 7.4, or nonimmune rabbit serum in a dilution of 1:100 was applied to the sections in both methods. In the immunocytochemical study, preparations were obtained from surface curetting of CU from 45 patients with squamous-cell CCU and dysplasias of CU, 21 patients with endocervicitis and 15 with colpitis, and also from 37 patients with benign and malignant tumors of other organs and without pathology of CU, and 12 clinically healthy women. Some of the material was used for traditional cervical cytology, and from the rest, preparations were made

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TABLE 1. Presence of ASCCU in Specimens of Various Lesions of the Squamous-Cell Epithelium of the Cervix Uteri and the Normal Cervix, Detected Immunohistochemically

No. of group	Material studied	Number of cases studied	Number of positive tests for presence of ASCCU	
1	Normal squamous epithelium of CU	10	2	
2	Dysplasias of II-III degree of severity	5	5	$p_{2-1}>0{,}9995$, p_{2-3} —n.s. p_{2-4} — n.s.
3	Squamous-cell CCU in situ	5	5	$p_{3-1}>0{,}9995$, $p_{3-5}>0{,}9995$, p_{3-2} —n.s. p_{3-4} —n.s.
4	Invasive forms of squamous-cell CCU	40	40	$p_{4-1}>0{,}975$, $p_{4-5}>0{,}99$
5	Adenocarcinoma of CU	2	0	

Legend. Here and in Table 2: n.s. denotes difference not significant.

TABLE 2. Presence of ASCCU in Cervical Films Obtained from Different Groups of Women with Pathology of and a Normal Cervix Uteri, Detected Immunocytochemically

No. of group	Clinical diagnosis	Number of cases studied	Number of positive tests for presence of ASCCU	
1	Clinically healthy women	12	0	
2	Normal squamous epithelium of CU in women with various tumors in other locations	37	5 (13,5 %)	$p_{2-5}>0{,}9995$, $p_{2-6}>0{,}9995$, $p_{2-7}>>0{,}9995$.
3	Colpitis	15	0	$p_{3-5}>0{,}9995$, $p_{3-6}>0{,}9995$, $p_{3-7}>>0{,}9995$, $p_{3-1}>$—n.s.
4	Endocervicitis	21	1 (4,8 %)	p_{4-1} —n.s. $p_{4-5}>0{,}9995$, $p_{4-6}>>0{,}9995$, $p_{4-7}>0{,}9995$.
5	Dysplasia of Cu of the II-III degree	21	13 (61,9 %)	$p_{5-1}>0{,}9995$, p_{5-6} —n.s. p_{5-7} —n.s.
6	Preinvasive forms of CCU (in situ and stage I^a)	18	13 (72,2 %)	$p_{6-1}>0{,}9995$, p_{6-7} — n.s.
7	Invasive forms of CCU (I^b, II, III)	15	13 (86,6 %)	$p_{7-1}>0{,}9995$.

for immunocytochemical investigation. Preparation of these films, and also the way of obtaining polyclonal antibodies to ASCCU were described by the writers previously [1, 2]. The reaction was assessed as positive if the preparation contained more than 5% of fluorescent cells, and the degree of intensity was determined on the scale of −, ±, and ++. The significance of differences in the frequency of detection of ASCCU in the different groups of women was estimated on a "Toshiba T-1200" IBM-compatible personal computer, using the chi-square test.

EXPERIMENTAL RESULTS

The results of the immunohistochemical investigation are given in Table 1. In both IFT and IPM, on treatment of tissue sections with antibodies to ASCCU, it was detected in all specimens of squamous-cell CCU. The same pattern also was observed in a group of patients with dysplasias of different degrees of severity and in the group of patients with CCU in situ, but the reaction was weak in intensity compared with invasive forms of CCU. In two of the cases of adenocarcinoma of CU studied, the antigen was not detected. A distinct reaction was observed in two of 10 samples in normal squamous epithelium of CU. As the results of statistical analysis in Table 1 show, the difference between the frequency of discovery of ASCCU in normal squamous cervical epithelium was significant ($p_{2-1} > 0.9995$, $p_{3-1} > 0.9995$, $p_{4-1} > 0.975$). Incidentally, tumors which, by their morphologic picture, were identified as highly differentiated, contained more ASCCU than tumors at a low level of differentiation. In normal squamous cervical epithelium a positive reaction was obtained mainly in prickle cells and the intensity was lower than in specimens of CCU. In a number of areas of squamous-cell metaplasia of the cervical epithelium which were studied, a weak positive reaction also was observed. The results of a study of tissue

sections of CU were confirmed by the immunocytologic method of detection of ASCCU in cervical curettings. The results of IFT conducted on films from 139 women, shown in Table 2, were compared with the diagnosis obtained on the basis of a comprehensive study: clinical, histologic, and cytologic. ASCCU were absent in films from clinically healthy women, but in the group of patients with tumors in other situations and without pathology of CU, a positive reaction for ASCCU was observed in 17% of cases. The highest rate of detection of this marker was observed in the group of patients with clinically well defined forms of squamous-cell CCU (86.6%): with initial forms of CCU (72.2%), and in patients with dysplasias of CU of the II-III degree (61.9%). When these groups were compared with clinically healthy women, and also with patients with various diseases of other organs but without pathology of CU, the difference in the frequency of detection of ASCCU was significant ($p_{5-1} > 0.9995$, $p_{6-1} > 0.9995$, $p_{7-1} > 0.9995$). A significant difference also was found in the content of ASCCU when groups of patients with dysplasias of CU and CCU were compared with groups of patients with predisposing diseases of CU (endocervicitis and colpitis) (Table 2), whereas if the latter were compared with the group of clinically healthy women and patients without pathology of CU, but with other diseases, the difference was not significant (Table 2). On comparison of data obtained for the group with dysplasias of CU and with preinvasive and invasive CCU, no significant difference likewise was found. This last state of affairs imposes definite restrictions on the use of ASCCU in the differential diagnosis between dysplasias and initial forms of CCU.

A similar picture of an increase in the content of the tumor-associated marker in the cells depending on the development of dysplasia of CU or CCU, was demonstrated by Japanese workers who studied the TA-4 antigen, which is currently widely used as a prognostic factor in tumor regression [8, 9]. When the trend of precancerous lesions of an organ is to be discovered, and when the efficacy of treatment and the course of a malignant disease has to be monitored, or subclinical recurrences and metastases identified, information obtained by the use of immunologic markers can be very important. Probably an essential criterion for the choice of such markers is correlation between the level of its expression and the degree of progression of the disease. Since the concentration of tumor-associated marker in the blood serum depends on its production and secretion by the tumor, its metabolism in the body, and the presence of tumor-nonspecific cross-reacting substances in the serum, before we can begin to determine it in the blood, we must first of all discover the degree of expression of the antigen in the given type of tumor and correlate its dependence on the clinical stage of the disease and its association with progression of the process [6]. The results of the immunocytohistochemical investigation described above point to the presence of correlation between the level of expression of ASCCU and an increase in severity of dysplastic changes in the epithelium of CU, and also with the onset of invasive growth in CCU. This antigen can accordingly be regarded as a tumor-associated marker, which may have a role primarily in prognosis, in the dynamic observation of patients with severe dysplasias and with CCU.

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