SURFACE ANTIGENS FROM LARYNGEAL PAPILLOMA CELLS

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Cells of laryngeal papillomas occurring in children contain a specific surface antigen (or antigens). Humoral antibodies against this antigen have a cytotoxic action on the papilloma cells.

Cells of experimental tumors induced in animals contain tumor-specific transplantation antigens [2-6, 9, 10]. Antigens located on the cell surface are found in human tumors, and these also evidently are tumor-specific [1, 7].

The object of this investigation was to study antigens on the cell surface in laryngeal papillomas in children.

EXPERIMENTAL METHOD

A suspension of papilloma cells was obtained by trypsinization of the tumor on the day of its removal. The number of dead cells in the suspension varied from 2 to 15%. As a rule, tests with the cells of one tumor were carried out with autologous and homologous patients' sera, and with healthy human serum as the control. The sera were chosen from persons of the same blood group as the donor of the tumor. Cells from the mucous membrane of the palate, taken from children during tonsillectomy, also were investigated.

Analysis of the cell surface antigens was carried out by the indirect method of fluorescent antibodies in Möller's modification [8]. Rabbit anti-human γ-globulin, conjugated with fluorescein isothiocyanate, was used in the reaction. Tests were carried out with 500,000 cells. The index of fluorescence (IF) was calculated by comparing the experimental and control results, using Klein's formula [6]; the difference obtained by subtracting the percentage of unstained cells in the experiment from the percentage of unstained cells in the control, divided by the percentage of unstained cells in the control.

The cytotoxic action of the patients' sera on papilloma cells was investigated in the following modification of this test: 0.4 ml of serum heated to 58° for 20 min was added to 250,000 cells and the mixture incubated for 1 h at 37°. Next, 0.4 ml complement (guinea pig serum) was added to the suspension of cells in the serum, and the mixture was incubated for 1 h 20 min at 37°. The suspension of cells was centrifuged for 5 min at 300 g. The cells were stained and the index of cytotoxicity (IC) calculated by the formula: difference obtained by subtracting the percentage of living cells in the experiments from the percentage of living cells in the control, divided by the percentage of living cells in the control.

Absorption of 0.4 ml of patients' serum by 6 million homologous tumor cells or normal mucosal cells was carried out for 1 h at room temperature, and then for 15 min at 37°.

EXPERIMENTAL RESULTS

Tests of papilloma cells by the indirect method of fluorescent antibodies with autologous and homologous patients' sera revealed an antigen (or antigens) located on the cell surfaces. The antigen showed itself either as a ring of fluorescence around the edges of the cell or as points of fluorescence distributed

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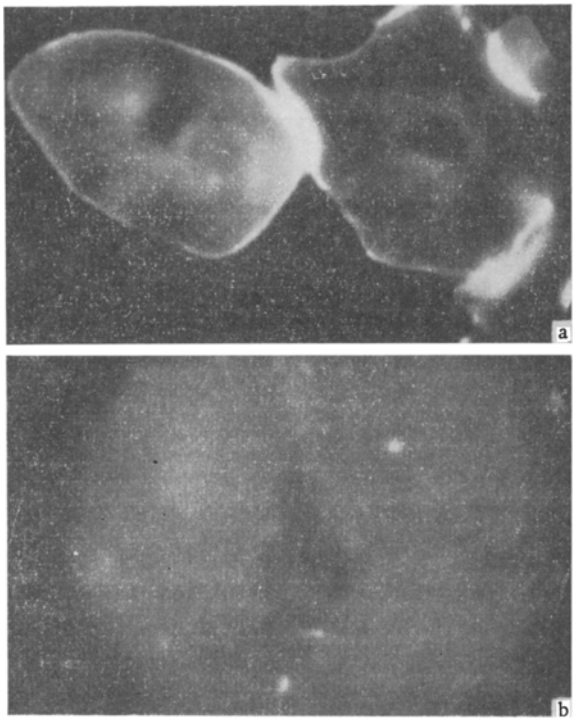

Fig. 1. Cell surface antigen from laryngeal papillomas in children. A) Reaction with serum of affected child; B) reaction with healthy serum. Indirect method of fluorescent antibodies, 90×.

diffusely over the surface of the cells (Fig. 1). Results obtained in immunofluorescence tests with the cells of 30 tumors are given in Table 1. It is clear from Table 1 that the percentage of fluorescent cells was higher in tests with sera from patients with papillomatosis than with normal sera. Cells of normal mucous membrane from 14 children gave a much smaller percentage of fluorescence. The IF in 45% of tests of papilloma cells with autologous and homologous sera was above 0.3. After absorption of the patients' sera with papilloma cells the percentage of fluorescence of the papilloma cells was considerably reduced, whereas absorption of these sera with normal cells caused virtually no change in activity of the sera (Table 2). Absorption of the patients' sera with normal cells yielded sera which did not react with the control cells but which produced fluorescence of antigen of the papilloma cells.

Because of the discovery of a surface antigen of the papilloma cells it was essential to discover whether the immunologic tests can cause death of the tumor cells. The results of experiments performed to study 36 tumors are shown in Table 1. The patients' sera exerted a cytotoxic action on the papilloma cells. Control sera had no such effect. In 75% of tests with autologous sera and in 80% with homologous sera, the IC was above 0.3. Absorption of the sera by papilloma cells sharply reduced the cytotoxic action on the tumor cells, whereas absorption of the sera by cells of normal mucous membrane caused no change in the cytotoxic action of the sera (Table 2). Absorption by human embryonic cells likewise did not abolish the cytotoxic effect. The results of immunofluorescence and cytotoxicity tests with the same cells and with patients' sera were very similar. However, the percentage of reacting tumor cells in the immunofluorescent test with normal sera was higher than the percentage of reacting cells in the cytotoxicity test with these sera. For this reason, the IF was lower than the IC. No connection could be found between the clinical picture of the disease and its immunologic characteristics.

The results demonstrate the existence of a tumor antigen (or antigens) localized on the surface of cells of laryngeal papillomas in children.

TABLE 1. Detection of Surface Antigen of Laryngeal Papilloma Cells from Children

Method	Cells	Sera	No. of sera	Percent of reacting cells				
				0-20	21-40	41-60	61-80	81-95
Immunofluorescence	Papillomatous	Autologous	30	3	8	8	5	6
		Homologous:						
		patients'	44	6	11	15	8	4
		healthy	26	14	8	3	1	
	Normal	Autologous	14	12	2			
		Homologous:						
		patients'	19	15	4			
		healthy	3	2	1			
Cytotoxicity	Papillomatous	Autologous	36	3	8	12	7	6
		Homologous:						
		patients'	41	1	5	16	15	4
		healthy	31	20	10	1		

TABLE 2. Immunofluorescence and Cytotoxicity Tests with Absorbed Sera

Method	Expt. No.	Percent of reacting cells before absorption	Percent of reacting cells after absorption by papilloma cells	Percent of reacting cells after absorption by normal cells
Immunofluorescence	1	56	7	38
	2	28	2	0
	3	93	2	80
	4	–	15	46
	5	40	20	34
	6	90	–	82
	7	32	14	32
Cytotoxicity	1	66	30	68
	2	68	19	72
	3	71	22	–
	4	57	–	54

Legend: (–) test not carried out.

This antigen is absent from normal mucous membrane cells. Humoral antibodies are synthesized against it in the body. In the cytotoxicity test, patients' sera exhibit activity against the tumor cells. It can be postulated that the antigen thus demonstrated is a tumor-specific antigen of transplantation type. The possibility likewise is not ruled out that it is a virus located on the surface of the tumor cell, or an antigen induced by an infective or tumorigenic virus.

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