ANTIGENS OF ROUS VIRUS PARTICLES IN VIRUS-FREE TUMORS INDUCED IN ADULT RATS

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In approximately 50% of rats with primary "virus-free" tumors induced by Rous virus (Schmidt-Ruppin strain), a cellular response of immunity to transplantation antigens of these tumors was found. Meanwhile, group-specific antibodies were not present in the blood sera of these animals nor antigens of the virus particle in the tumors.

A previous investigation [4] showed that tumors induced in adult outbred Wistar rats by Rous virus of the Schmidt-Ruppin strain can be virogenic, "virus-free," and in very rare cases they may contain virus in the mature form. Particular interest is attached to the "virus-free" tumors in which the synthesis of Rous virus could not be activated by the artificial heterokaryon method or by x-ray irradiation.

The object of the present investigation was to study antigens of the virus particle by the fluorescent antibody method (FAM) and complement-fixation test (CFT) in "virus-free" tumors induced in adult Wistar rats by Rous virus of the Schmidt-Ruppin strain, and also to study the factors of cellular and humoral immunity against antigens of the virus particle and against transplantation antigens of these tumors.

EXPERIMENTAL METHOD

Adult outbred Wistar rats with primary tumors induced by Rous virus of the Schmidt-Ruppin strain were used in the experiments.

Antigens of virus particle. To determine antigens of the virus particle in the rat tumors, hyperimmune hen sera against extract from Rous fowl sarcoma were used. According to Sovova and Klement [11], these sera contain antibodies against type-specific and group-specific (GS) antigens of the virus particle.

These antigens were determined by the direct fluorescent antibody method in cultures of rat tumor cells fixed with acetone. Controls of the specificity of fluorescence were described previously [6]. The results of the FAM were expressed in two ways: as the percentage of fluorescent cells and as the fluorescence index. The percentage of fluorescent cells was regarded as significantly different from the control if the corresponding fluorescence index was greater than 0.2 [10]. Virus GS-antigens in the rat tumors were determined simultaneously by the CFT. The sera of rats and hamsters with "virus-free" tumors induced by Rous virus, not containing virus-neutralizing antibodies, were used as the source of antibodies.

Antibodies against antigens of the virus particle. Virus-neutralizing antibodies in rats with tumors were determined by the virus neutralization reaction [3]. The results of this test was expressed as neutralization indices, which were regarded as negative if their value was less than 2 [5]. Antibodies against GS-antigens of the rat tumors were determined by the CFT [2]. Sera were regarded as negative if their titers in the CF 2 were less than 1:10.

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TABLE 1. Investigations of Virus Particle Antigens in Tumors and Factors of Cellular and Humoral Immunity

Rat No.	Antigens of virus particle in tumors (FAM, % of fluorescent cells)*	GS-antigens in rat tumors (CFT)	Virus-neutralizing antibodies in rats' sera (neutralization index)	GS-antibodies in rats' sera (titers of antibodies in CFT)	Humoral cytotoxic antibodies (cytotoxic index)	Cellular reaction of immunity to transplantation antigens of rat tumors
1	6	Not found	0,8	—	0	—
2	61	» »	0 †	—	0,07	+
3	6	» »	1	—	0,05	+
4	9	» »	0,66	—	0,01	+
5	8	» »	0,36	—	0	—
6	7	» »	0,66	—	0,09	+
7	8	» »	0,66	—	0,09	—
8	15	» »	0,6	—	0,08	—
9	2	» »	0,3	—	0,07	+
10	8	» »	0 ‡	—	0	—
11	8	» »	0 ‡	Serum anticomplementary	0,11	—
12	6	» »	0,14	Ditto	0,12	+
13	26	» »	0 ‡	1:10	0,09	—
14	27	» »	0,7	1:10	0,08	+
15	9	» »	0,8	—	0,03	+
16	37	» »	0	1:10	0	—
17	7	» »	0,3	—	0,06	+
18	6	» »	0,8	—	0,05	—

* Percentage higher than 26 is significantly different from control (fluorescence index greater than 0.2).

† Test not carried out.

‡ Not different from control.

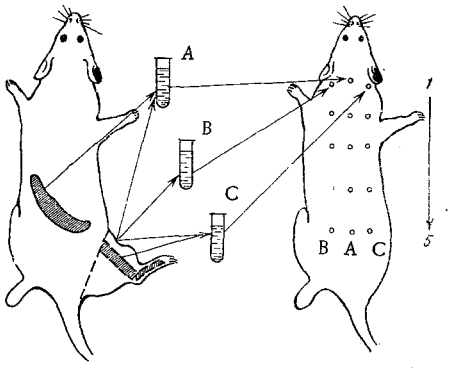

Fig. 1. Test of neutralization of tumor cells by lymphocytes (scheme): A, B, C) Suspensions injected (explanation in text); 1-5 sites of injection of 1:10 dilutions of tested cell suspensions.

Antibodies against transplantation antigens. Humoral antibodies were determined by the cytotoxic reaction in vitro as described by Gorer et al. [8], in B. D. Brondz's modification [1]. A cytotoxic index below 0.15 was regarded as negative. Factors of cellular immunity against transplantation antigens of the rat tumors were investigated by the test described by Yoshida et al. [12], based on neutralization of tumor cells by lymphocytes in an autologous system, in the writers' modification. The limb together with the tumor nodule, and the spleen were removed from rats with primary tumors induced by Rous virus and measuring not more than 2×2 cm, and at the same time the bone marrow was extracted from the diaphyses of the removed femur and tibia. Next, 4 rows of 1:10 dilutions of 3 suspensions of the following cells were prepared: A) rat tumor cells (5×10^6) and autologous splenic lymphocytes (3×10^8); B) rat tumor cells (5×10^6); C) rat tumor cells (5×10^6) and autologous bone marrow lymphocytes (3×10^8), or in some experiments (C_1), homologous normal lymphocytes from rats' spleens. The resulting dilutions of the 3 cell suspensions were incubated at 37° for 30 min and then injected subcutaneously into rats in the manner illustrated in Fig. 1. If growth of the tumor cells was inhibited by spleen cells taken in dilutions higher by 2 powers of 10 (2 log) than control suspensions B, C, or C_1, the result was interpreted as indicating a cellular response of the host to transplantation antigens of the rat tumors.

EXPERIMENTAL RESULTS

The various immunologic methods described above were used to investigate 18 rats with primary "virus-free" tumors induced by the Schmidt-Ruppin strain of Rous virus.

In 4 of the 18 rat tumors, antigens of the virus particle were detected by means of the FAM in 26-61% of tumor cells (Table 1). In the CFT, no GS-antigens were found in any of the rat tumors. This was probably because antisera were used in the CFT in a low titer (1:20).

In 3 of the 18 sera from rats with tumors, GS-antibodies were found in the CFT. The titers of these sera were 1:10 (Table 1). No virus-neutralizing activity was found in any of the 16 investigated sera from rats with tumors.

In a parallel series of tests, using an autologous system, factors of cellular and humoral immunity to transplantation antigens of primary tumors induced by Rous virus were studied in the sera of rats with these tumors. The results of the neutralization reaction of tumor cells by lymphocytes showed that following transplantation of suspension A, inhibition of growth of tumor cells was found in 9 of 18 cases (2 log., in 1 case 3 log.), compared with transplantation of suspensions B, C, or C_1. In 3 of the 18 cases (rats Nos. 8, 13, and 18), the degree of inhibition was 1 log. In 2 cases (rats Nos. 2 and 8), inhibition of growth (2 log.) of the experimental A and control C_1 graft of tumor cells was observed under the influence of normal homologous lymphocytes from rats' spleen. Under similar experimental conditions, lymphocytes from bone marrow had no cytopathogenic action.

None of the 14 sera from rats with tumors induced by Rous virus, when tested by the cytotoxic reaction in vitro, possessed any obvious cytotoxic effect against autologous cells of these tumors (Table 1).

Hence, the cellular reaction of immunity against transplantation antigens of "virus-free" tumors of rats was found in approximately 50% of animals, but no connection was demonstrated either with antigens of the virus particle in the tumors or with the presence of antibodies against those antigens in the animals' sera.

Cellular antigens detected by the transplantation test in a system of tumors induced by Rous virus are known not to be antigens of the virus particle, but antigens specific against the virus inducing their synthesis [7, 9]. A study of the factors of cellular immunity in rats with "virus-free" tumors, in which no antigens of the virus particle are found, and also no reaction of the host against them is present, could contribute toward the elucidation of the specific nature of these tumors.

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