CHANGES IN THE ONCOGENIC PROPERTIES OF MURINE RETICULOSARCOMA PRODUCED BY FREUND'S COMPLETE ADJUVANT

S. P. Gordienko and A. I. Ageenko

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Ten days after receiving an injection of Freund's complete adjuvant into the plantar pad of the hind limb, Wistar rats were inoculated with a suspension of cells of a type 321-KRS murine reticulosarcoma by subcutaneous injection in the dorsal region. In rats previously receiving Freund's complete adjuvant tumors were found not at the site of subcutaneous injection of the 321-KRS sarcoma cells, but intraperitoneally, a result never observed in the group of animals not receiving the adjuvant. The mean latent period of tumor development was considerably prolonged. Freund's adjuvant, when injected simultaneously with cell-free extract of 321-KRS sarcoma tissue into newborn Wistar rats, was found to stimulate carcinogenesis.

Recent work has shown that Freund's adjuvant (FA), widely used in experimental practice to increase immunological reactivity, can also stimulate carcinogenesis due to viruses [4-7, 10].

In the investigation described below the effect of FA was studied on the growth of transplanted tumor material and on the carcinogenicity of cell-free extracts of 321-KRS murine reticulosarcoma, induced primarily by a cell-free saline extract from human reticulosarcoma tissue [1].

The result of experimental studies of this neoplasm (the neoplastic activity of RNA isolated from sarcoma 321-KRS tissue, and also the results of virological, immunological, and physicochemical analysis) suggest that this tumor is virogenic in nature [3].

EXPERIMENTAL METHOD

Strain 321-KRS of murine reticulosarcoma was used in the investigation. After subcutaneous inoculation of young Wistar rats with cell suspensions, this tumor grows comparatively quickly and takes successfully in 100% of cases. This neoplasm can be induced by a cell-free extract from sarcoma 321-KRS tissue in Wistar rats in 13.3% of cases after a mean latent period of 13 months [1].

Cell-free extract of sarcoma 321-KRS were prepared as follows. The tumor was minced under sterile conditions on ice, then ground in a mortar for 10-15 min in physiological saline in the ratio of 1:3. The mince was then frozen to −70°C and thawed three times. The resulting suspension was centrifuged twice at 2500 rpm for 20 min. The supernatant was tested for absence of cells by staining with trypan blue. The cell-free extract was injected into Wistar rats a few days old, in a dose of 0.5 ml, intraperitoneally.

The cell suspensions obtained by trypsinization, and the cells were counted in a Goryaev's chamber. Month-old Wistar rats received a dose of 3.5×10^5 cells, rats aged 4.5 months a dose of 3.5×10^6 cells.

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TABLE 1. Effect of Freund's Adjuvant on Growth of Transplanted Murine Sarcoma 321-KRS

Material inoculated	Age of Wistar rats at moment of inoculation of tumor (in months)	Dose of sarcoma 321-KRS cells	Number of rats		Mean incubation period (in days)	Location of tumor	
			total number inoculated	number with tumor		at site of injection	intraperitoneally
Fruend's adjuvant*	1	–	15	–	–	–	–
Sarcoma 321-KRS cells + Fruend's adjuvant	1	3.5×10^5	30	30	90	4	26
Sarcoma 321-KRS cells (control)	1	3.5×10^5	30	4	17	4	–
Freund's adjuvant + sarcoma 321-KRS cells...	$4^1/_2$	3.5×10^6	20	13	160	–	13
Sarcoma 321-KRS cells (control)†	$4^1/_2$	3.5×10^6	20	2	22	2	–
Fruend's adjuvant	$4^1/_2$	–	15	–	–	–	–

* Fruend's adjuvant was injected into the plantar pad of the hind limb 10 days before inoculation of the sarcoma 321-KRS cells.
† Sarcoma 321-KRS cells inoculated subcutaneously in the dorsal region.

The formula of the FA used in the investigation specified 10 mg killed tubercle bacilli to 1 ml of a 50% oil-and-water emulsion and the product was injected into adult animals into the plantar pad of the hind limb in a dose of 0.4 ml. For the newborn rats, Freund's complete adjuvant (Difco, USA) was used in a dose of 0.1 ml.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of FA on growth of tumor cells of sarcoma 321-KRS was investigated. As Table 1 shows, in the control group of month-old animals (not receiving FA) neoplasms, located subcutaneously, developed in only 4 of the 30 rats after a mean latent period of 17 days. No intraperitoneal tumors were found. In the experimental group of rats, sarcoma cells injected in a dose of 3.5×10^5 subcutaneously into the dorsal region induced the growth of tumors in month-old Wistar rats, inoculated with FA 10 days beforehand, in 100% of cases. In 26 of the 30 rats no neoplasms were found at the site of injection, but tumors were located intraperitoneally, where they reached a diameter of 3-8 cm. The mean latent period of appearance of these tumors was 90 days. In four rats of this group neoplasms appeared at the site of inoculation after a latent period of 17 days, while in two animals tumors developed under the dorsal skin and intraperitoneally. Hence, in rats receiving FA the latent period of appearance of the reticulosarcomas was considerably lengthened, and second, the tumors were not located at the site of subcutaneous injection of the 321-KRS sarcoma cells, but intraperitoneally, which was never observed in the control series (in which the percentage of successful takes was very low, namely 12%, compared with 100% in the experimental group).

A similar effect of FA on the oncogenic properties of reticulosarcoma KRS-321 was observed in the experiments of series II on Wistar rats aged 4.5 months. The results of the experiments are given in Table 1. They show that sarcoma 321-KRS cells, in a dose of 3.5×10^6, when injected subcutaneously into the dorsal region of animals previously receiving FA, induced intraperitoneal tumors in 13 of 20 animals (65%) after a mean latent period of 160 days. In one case a tumor was found 270 days after injection of the tumor cells. In the control group of rats, neoplasms appeared subcutaneously in two of the 20 rats after a latent period of 22 days. In the experiments of both series I and series II, neoplasms which developed intraperitoneally were reticulosarcomas in their histological structure.

In the experiments of series III, the allogeneic transplantability of sarcoma 321-KRS cells was studied in noninbred rats and inbred rats of the August line, receiving a preliminary injection of FA. In no case (altogether 30 noninbred and 40 August rats were inoculated) did tumors appear. The animals remained under observations for over a year.

TABLE 2. Effect of Freund's Adjuvant on the Oncogenic Activity of Cell-Free Extracts from Sarcoma 321-KRS Tissue

Material inoculated	Material used to prepare extracts	Age of Wistar rats at moment of inoculation of tumor (in months)	Number of rats		Mean incubation period (in days)	Location of tumor
			total number inoculated	number with tumor		
Fruend's adjuvant	–	1	20	–	–	–
Fruend's adjuvant + cell-free extract	Intraperitoneal tumor appearing after inoculation of sarcoma 321-KRS cells into rats previously treated with Freund's adjuvant	1	95	44	136	At site of injection
Cell-free extract		1	26	–	–	

Having regard to the hypothesis that this neoplasm is virogenic in nature, experiments were carried out to investigate the effect of FA on the oncogenic activity of cell-free extracts* of reticulosarcoma 321-KRS.

Cell-free extract from the reticulosarcoma tissue, when injected into newborn Wistar rats, did not give rise to neoplasms in any of the animals used (Table 2). However, in the group of rats inoculated with FA simultaneously with the cell-free extract, tumors developed in 46% of animals after a mean latent period of 136 days. At autopsy on these rats, neoplasms up to 8 cm in diameter were found all over the peritoneal cavity, invading the viscera, very firm in consistency, and associated with large quantities of hemorrhagic ascites fluid. In their histological structure they were polymorphocellular reticulosarcomas.

These results indicate that injection of Freund's complete adjuvant has a significant effect on the biological properties of reticulosarcoma 321-KRS. After subcutaneous inoculation with cell suspension, tumors appeared after longer intervals, and they were located not subcutaneously, but intraperitoneally. With an increase in the animal's age, the tumors appeared after a longer latent period and in a smaller proportion of cases.

It is important to note that no intraperitoneal tumors were observed in animals untreated with FA when inoculated with sarcoma 321-KRS cells subcutaneously in the dorsal region, despite the fact that these subcutaneous inoculations of the neoplasm were repeated over many years and over more than 100 generations. One of the writers (S. P. Gordienko) has previously shown that tumors of different etiology, for instance sarcomas induced with 20-methylcholanthrene (including in animals receiving FA), when injected subcutaneously never yielded intraperitoneal growths.

The following mechanism of translocalization of tumor growth can be postulated: by activating the cellular factors of antitumor immunity, FA led to the death of subcutaneously transplanted 321-KRS sarcoma cells, but the hypothetical murine oncogenic virus, existing in an integrated state with the cell genome, induced intraperitoneal tumors. Possibly for this reason the latent period of development of the tumors was lengthened. The increase in oncogenic activity of this virus was facilitated by humoral antibodies, the more rapid synthesis of which was also stimulated by FA in accordance with a phenomenon of the Kalliss [9] type. If this hypothesis is correct, attempts can be made to use complete FA as an instrument for liberating oncogenic virus existing in an integrated state with the cell genome.

The results described thus show that FA, when injected simultaneously with cell-free extracts from sarcoma 321-KRS tissue into newborn Wistar rats stimulated tumor growth in a similar manner to that demonstrated previously on other models of virus carcinogenesis [4-7, 10].

*The presence of whole tumor cells in the extracts was ruled out by the triple freezing and thawing at −70°C. In addition, each specimen prepared was examined under the microscope to confirm the absence of cells.

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