Dynamics of Tumor Growth in Brattleboro and Wag Rats Injected with Walker 256 Carcinosarcoma Cells in Different Doses

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The peculiarities of Walker 256 tumor growth depending on the dose of transplanted cells were studied in rats differing by vasopressin production. Transplantation of 10^5 cells does not lead to tumor development. The dose of 7×10^5 cells induces progressively growing tumors in WAG rats, while in Brattleboro rats tumors regressed after a short period of growth. Increasing the dose to 2.8×10^6 cells was inessential for the dynamics of tumor growth in WAG rats, but stimulated regression of tumor growth in Brattleboro rats, producing no vasopressin. This dose dependence suggests the involvement of the immune system into the dynamics of tumor growth.

Key Words: *Walker 256 carcinosarcoma; vasopressin; immunity*

Strain-nonspecific Walker 256 carcinosarcoma is characterized by sensitivity to the effects of hormones, in particular to those mediated by regulation of Rho-mediated signal cascade [9]. Walker 256 tumor grows slowly in Brattleboro rats genetically incapable of vasopressin production compared to normal WAG rats [6]. The hormone imbalance of Brattleboro rats involving primarily the mechanisms of kidney functioning is also essential for the parameters of the immune system and forms a condition with reduced level of antibody production [1,5].

We studied dose dependence of tumor growth after Walker 256 carcinosarcoma cell injection to Brattleboro and WAG rats.

MATERIALS AND METHODS

Walker 256 tumor strain maintained *in vivo* was used in the study. Tumor cell suspension was injected with a syringe into the thigh muscles to 6-month-old WAG and Brattleboro rats (200-250 g; n=30) in doses of 10^5, 7×10^5, and 2.8×10^6 cells. This dose range was selected on the basis of published reports [11]. After 7 days, the tumors were measured with a slide gage in three perpendicular directions and tumor volume was estimated. The level of vasopressin was measured by reverse phase microcolumn chromatography (Milichrome) of neuropituitary extracts, hormone production was evaluated indirectly by urine osmolality on an MT-2 osmometer (Burevestnik Firm) and mean daily water consumption [4]. Functional activity of peripheral blood macrophages was evaluated by the reaction of 24-h culture of peritoneal cells to opsonized sheep erythrocytes after intraperitoneal injection of saline or recombinant TNF-α (1×10^5 U/200 g) [2]. Differential leukocyte count was evaluated on fresh blood smears prepared from the blood collected from the carotid artery. Smears dried on air were fixed (1 min) in methanol and stained routinely by the method of Romanowskii—Giemsa. Cells were counted and expressed in percent of total leukocyte count.

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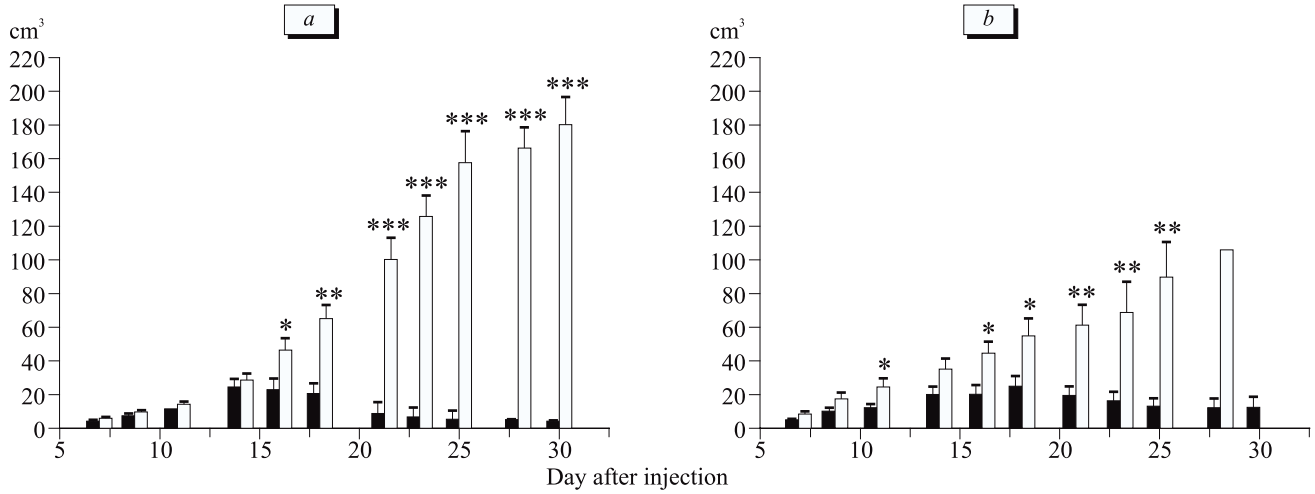

Fig. 1. Time course of tumor growth in WAG (light bars) and Brattleboro rats (dark bars) after injection of Walker 256 carcinosarcoma cells in a dose of 7×10^5 (*a*) and 2.8×10^6 cells per animal (*b*). *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to Brattleboro rats.

The significance of differences was evaluated using Student's *t* test for independent variables.

RESULTS

Neurohormone vasopressin is a key regulator of water metabolism. Water consumption and concentration of urine are determined by the intensity of vasopressin secretion. Blood level of vasopressin in WAG rats corresponded to normal, while in Brattleboro rats no hormone was detected (Table 1) [4]. Despite different hormonal profiles, not a single case of solid tumor formation after injection of carcinosarcoma cell suspension in a dose of 10^5 cells was detected in any of the groups. Increasing the dose of Walker 256 cells to 7×10^5 led to the formation of tumor nodes at the site of injection, their growth dynamics was strain-specific (Fig. 1, *a*). The tumors progressively grew in WAG rats, their size linearly depended on the time elapsed after inoculation. In Brattleboro rats, similar growth was observed over 15 days, but then the tumor regressed and completely disappeared. Further 4-fold increase in the dose of injected cells (to 2.8×10^6) did not change the dynamics of tumor growth in WAG rats, but led to a certain decrease in the mean size of tumors, presumably, because of mortality of animals with large tumors. By day 30 of the experiment, all animals of this group died. In Brattleboro rats, this increase in the dose reduced the intensity of tumor growth and stimulated tumor regression (Fig. 1, *b*).

The same threshold concentration for tumor induction (at least 10^5 cells) in transplantation of Walker 256 carcinosarcoma in WAG and Brattleboro rats can indicate similar mechanisms of natural

TABLE 1. Physiological and Morphological Differences between WAG and Brattleboro Rats

Parameter	WAG	Brattleboro
Vasopressin, picomol/neuropituitary	990±89	0**
Water consumption, % of body weight	4.8±1.9	69.5±4.4**
Urine osmolality, mosm/kg water	1922.8±78.4	184.1±19.2**
Activity of peritoneal macrophage		
after saline injection	29.2±2.8	22.2±1.3
after TNF-α injection	43.4±2.5	27.6±3.4*
Neutrophils, %	9.6±1.9	30.8±3.8**
Monocytes, %	5.6±1.3	11.0±0.9*
Lymphocytes, %	79.4±2.4	49.8±4.4**
Leukocytes, $10^3/mm^3$	3.2±1.2	4.5±1.2

Note. Data on 6-10 animals are presented for each parameter. *$p<0.01$, **$p<0.001$ compared to WAG rats.

immunoresistance and early inducible response realizing for rapid rejection irrespective of vasopressin production [3]. The number of phagocytic macrophages in rats did not differ from that in controls injected with saline, though the sensitivity to stimulation with exogenous TNF-α varied. The time and dose dependence of tumor growth after transplantation of carcinosarcoma cells in higher concentrations corresponded to the typical time course of specific immune response only in Brattleboro rats. This can be partially due to leukocyte composition of the blood. Differential blood count revealed significant differences between WAG and Brattleboro rats (Table 1). The percent of neutrophils and monocytes is significantly higher in Brattleboro rats, while the lymphocyte fraction is less and the total leukocyte count in the blood is about the same in this rat strain. On the whole, specific immunity of Brattleboro rats (presumably, primarily its cell component) functions more effectively towards foreign tumors than in WAG rats. It is known that induction of natural killer cells by cytokines is a key factor of antitumor resistance [7]. This process, in turn, is sensitive to hormones [10]. The main hormonal difference between WAG and Brattleboro strains is the capacity to produce vasopressin [4]. It can be hypothesized that the absence of vasopressin or a physiological status of diabetes insipidus with high water exchange directly enhances immunogenicity of histocompatibility antigens in Walker 256 cells [8].

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