Variable response of spleen to Ehrlich's tumor according to the physical form (ascitic or solid) of the tumor[1]

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Summary. This study confirms the absence of splenomegaly in mice carrying Ehrlich's ascites; however, it reveals the presence of marked splenomegaly in mice bearing the same tumor in the solid state. Thus, the physical form of the tumor is an important factor when evaluating the host's response toward Ehrlich's tumor.

An increase in spleen weight is a common finding in tumor-bearing mice[4-20]. The splenomegaly is usually accompanied by a parallel decrease in thymus weight[5-8,16,21-26]. Both these phenomena are expressions of the host's response to the tumor. While thymic atrophy can be partially correlated with general cachexia[15-17,22,27], splenomegaly appears unequivocally as an active, hyperplastic process. However, 2 important exceptions to the 'splenomegaly rule' have been reported by Falk[3] and by El Hassan and Stuart[2]. These authors studied mice carrying Ehrlich and Landschütz ascitic tumors. They confirmed the thymic atrophy but were unable to find an increase in the size of the spleen.

The present study compares data concerning the relative spleen size in mice carrying the ascitic versus the solid form of Ehrlich's tumor.

Materials and methods. Tumor: Ehrlich's (ascitic) tumor (Heidelberg-Lettre strain) was injected i.m. to obtain the solid form, or i.p. to obtain the ascitic form.

Animals: 275 Swiss CF # 1 adult male mice, weighing 25–35 g, were used. All animals were kept in plastic cages and maintained on standard Purina Chow and water ad libitum.

3 groups were employed: 1. Mice inoculated i.p. with 10^7 ascitic cells in 0.3 ml of ascitic fluid to produce ascitic tumors. 2. Mice inoculated i.m. with the same number of cells and fluid volume in the posterior haunch to produce solid tumors. 3. Normal mice, as controls.

In a 1st set of experiments, the spleen weight was determined as the terminal stage of tumor evolution. 16 animals were sacrificed on the 15th day of Ehrlich's ascitic growth, while 30 mice were killed on the 23rd day of Ehrlich's solid tumor development. 30 mice provided controls for both groups.

In a 2nd set of experiments, 72 mice with Ehrlich's ascites and 77 with Ehrlich's solid tumors were sacrificed at 2-day intervals to determine the kinetics of changes in the spleen weight. 37 normal mice served as controls.

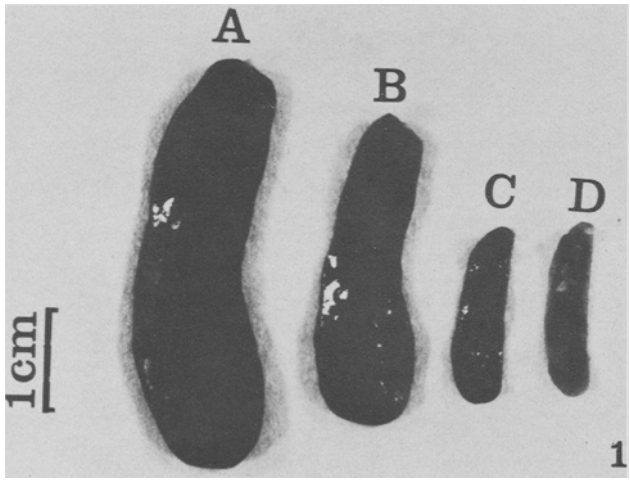

Fig. 1. A and B, examples of large spleens from mice carrying Ehrlich's tumor i.m. (solid); C, spleen from mouse control; D, spleen from mouse with ascitic Ehrlich's tumor.

An additional 3rd set of experiments was performed to test the influence of ascitic fluid upon the spleen size in mice already carrying a solid tumor. 13 mice were inoculated, i.m., in the same manner as those in group 2. 7 days later, they were reinoculated, i.p., with 10^7 ascitic cells in 0.3 ml of ascitic fluid. These animals were sacrificed on the 15th day following i.p. inoculation.

Mice with ulcerated tumors, those without tumors, as well as animals which died spontaneously were excluded from all experiments of this study. The final weight of the animal body was determined after the solid tumor was removed or the ascites had been drained. The weight of the spleens was reported as g per 100 g of b.wt. Samples of large and normal-sized spleens were examined histologically (H.E., Congo Red, Masson Trichrome, Reticulum, Giemsa, PAS) and tested for their RNA[28] and DNA[29] content.

Results. In the 1st experimental set, the average body weight after removal of the ascitic tumor was close to that of the controls. The weight of ascitic tumors (fluid + cells) was higher than that of solid tumors; however, after eliminating the fluid by centrifugation, the remaining cells represented approximately the same cellular mass as that of the solid tumors. The spleen was markedly enlarged in mice bearing the solid tumor (table 1, figure 1).

In the 2nd experiment, the increase in spleen weight was different for the ascites group as compared with the solid tumor group. In animals with i.p. tumors, the spleen showed a rapid enlargement followed by a rapid decrease in size. At the same time spleens of mice with solid tumors displayed a slower but continuous enlargement (figure 2).

Finally, in the 3rd experimental set, the splenomegaly of animals bearing solid Ehrlich tumors progressed up to a size similar to that of animals in group 2, despite the fact that these mice were also simultaneously carrying large Ehrlich ascitic tumors.

Microscopically, the spleens of mice carrying Ehrlich's ascites showed either a normal or a marked decrease in the number and size of lymphoid follicles parallel to an increase of medullary-type hematopoiesis in the red pulp (figure 3).

The spleens of mice bearing Ehrlich's solid tumors had large Malpighian follicles and increased medullary-type hematopoiesis in the red pulp, especially near the periphery

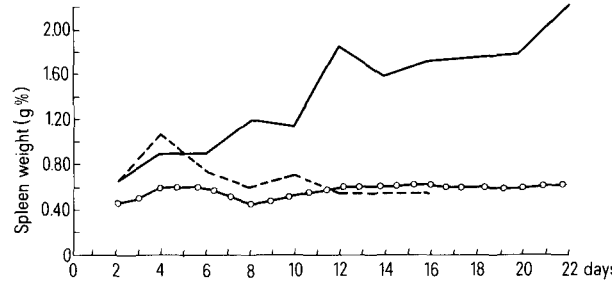

Fig. 2. Progression of spleen weight in mice with Ehrlich's ascitic tumor, Ehrlich's solid tumor and control. Solid (i.m.) tumor ——; fluid (i.p.) tumor - - - -; control (no tumor) O-O-O.

Table 1. Weight of spleen in mice with Ehrlich's ascitic tumors, Ehrlich's solid tumors, and control mice (mean weight/100 g of b.wt, SD and significance)

Group	No. of mice*	Spleen weight (%g)	Significance
Ascitic tumor	50	0.73±0.40	p=0.02
Solid tumor	50	1.94±0.66	p=0.0005
Solid and ascitic tumor	13	1.66±0.43	p=0.0005
Control	67	0.55±0.23	

* Mice from experiment 1 and 'end stage' tumors of experiments 2 and 3.

Table 2. Quantity of DNA and RNA per 100 mg of tissue in spleens of mice with Ehrlich's ascitic tumors, Ehrlich's solid tumors, and in normal controls (means and SD)

Group (No. mice)	DNA, mg	RNA, mg
Ascitic tumors (23)	11.7±2.23	5.68±0.91
Solid tumors (24)	11.2±1.41	5.84±0.76
Control (24)	12.1±1.38	5.76±1.35

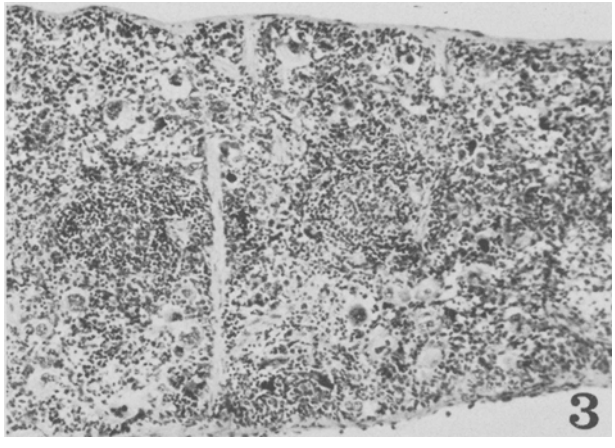

Fig. 3. Small spleen from a mouse carrying a 12-day-old Ehrlich's ascites. The lymphoid follicles remain located in the center. The rest of the spleen is occupied by medullary-type hematopoiesis. Note the abundance of megakaryocytes. (×40).

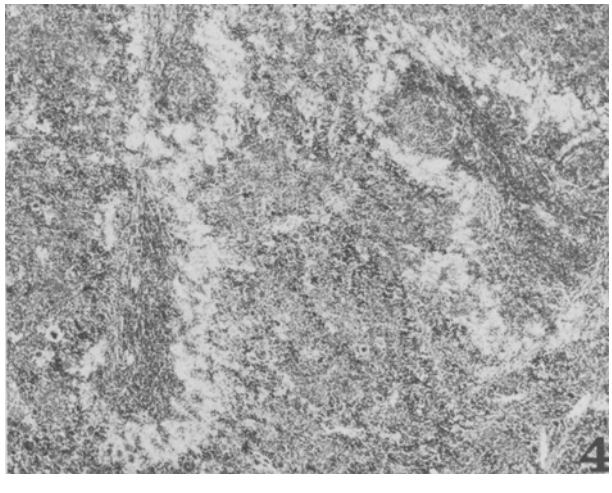

Fig. 4. Large spleen from a mouse bearing a 20-day-old Ehrlich's solid tumor. The lymphoid follicles are surrounded by wide coronas of collagenized-amyloid. (×40).

(subcapsular). The lymphoid follicles of these enlarged spleens were surrounded by a wide band of collagenized-amyloid tissue which stained rather poorly with Congo Red (figure 4).

The quantity of DNA and RNA per tissue unit of spleen was not altered in the larger 'solid-tumor-spleens' when compared to that of 'ascitic spleens' or normal spleens (table 2).

Discussion. The above study confirms previous observations regarding the absence of splenomegaly in mice carrying the ascitic form of Ehrlich's tumor. At the same time it demonstrates the presence of a marked splenomegaly in mice bearing the solid form of Ehrlich's tumor. Hence, the spleen in mice carrying Ehrlich's solid tumor reacts in the same way, namely, by splenomegaly, as it does in mice bearing any other solid tumor. The dissimilarity appears only in the case of the ascitic type of tumor growth.

The absence of splenomegaly during the development of ascitic tumors correlates with previous observations concerning the inability of mice bearing ascitic tumors to reject skin allografts[30,31]. The presence of splenomegaly in mice bearing Ehrlich's solid tumor also correlates with studies showing the persistence of the host's capacity to reject skin grafts[31]. One can infer that the factors which block the rejection of skin allografts may also preclude the development of splenomegally, since it is obvious that the presence or absence of splenic enlargement is related to the presence or absence of the ascitic fluid.

This is further enhanced by the temporary increase in spleen size (shown in figure 2), during the few days after i.p. inoculation, when ascitic cells grow into the peritoneal serosa as a temporary solid tumor. This initial splenomegaly reverts as soon as ascitic fluid accumulates. Nevertheless, the accumulation of ascitic fluid fails to arrest the course of the splenomegaly already initiated by the presence of a 7-day-old Ehrlich's solid tumor, as shown in our 3rd set of experiments. The size of the spleen in mice bearing a 7-day-old solid tumor is approximately equal to the size of the spleen in mice bearing a 4-day-old ascites.

It may, therefore, be concluded that the regression of the initial splenomegaly in mice developing the ascitic tumor is brought about by the arrest of the initial growth of the i.p. solid tumor which usually occurs after 4 or 5 days post inoculation. The persistence and increase of splenomegaly in hosts carrying both solid and ascitic forms of Ehrlich's tumor suggest, however, that the immunosuppressive activity of the ascitic fluid is not the essential element determining the absence of splenomegaly in the ascitic form. Purely physical factors such as dilution of toxic products or low level absorption of necrotic-toxic material derived from disintegrating, floating tumor cells may explain it.

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Cilia in stellate neurons of the rat cerebellum[1]

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Summary. Cilia in stellate neurons of the normal rat cerebellum are described. 8 cilia were observed in a total of 60 cells studied. An 8+1 pattern was found throughout their length. Furthermore, no arms, spokes or other accessory structures necessary for ciliary motion were seen. These findings make it possible to suggest that these cilia are probably without function and are related to the epithelial origin of these cells.

The presence of cilia in neurons was first described in the retinal rods and cones of kittens[3] and in the preoptic nucleus of the goldfish[4]. The existence of neuronal cilia was then related to cells performing either sensory or conducting functions[3,5,6], as well as to those possessing secretory activities[4,7,8]. They were later reported in different regions of the nervous system: peripheral autonomic ganglia[9,10], the spinal cord[11], inner neuronal layers of the retina[12], lateral geniculate nucleus[13], cerebellum[14] and cerebral cortex[15,16]. Although Karlsson[13] observed 1 cilium in each of 2 neurons which he serially sectioned and three-dimensionally reconstructed, which led us to believe that these are a common feature among neurons, cilia have otherwise been presented as a rare and occasional finding. This work deals with the frequent occurrence and particular morphology of cilia in stellate cells of the cerebellar cortex of the rat.

Material and methods. The molecular layer of the cerebellar cortex of 4 adult male Wistar rats selected at random was studied. Small tissue fragments of the cerebellar vermis, Larsell lobules 4–6, were obtained under ether anaesthesia of the animals[17]. Blocks were fixed according to the Kanaseki and Kadota method[18]. Details of this procedure have been described in a previous study[19].
5 tissue blocks from each rat were selected at random. 1 silver ultra-thin section of the molecular layer (external half) was chosen at random from each block and stained with uranyl-acetate and lead citrate. The ultrastructural identification of stellate neurons was made according to Palay and Chan-Palay[20]. A total of 60 cellular profiles (15 from each animal) were studied.
Results and discussion. 8 cellular profiles each possessing 1 cilium were found in a total of 60 cells (13.3%). There was

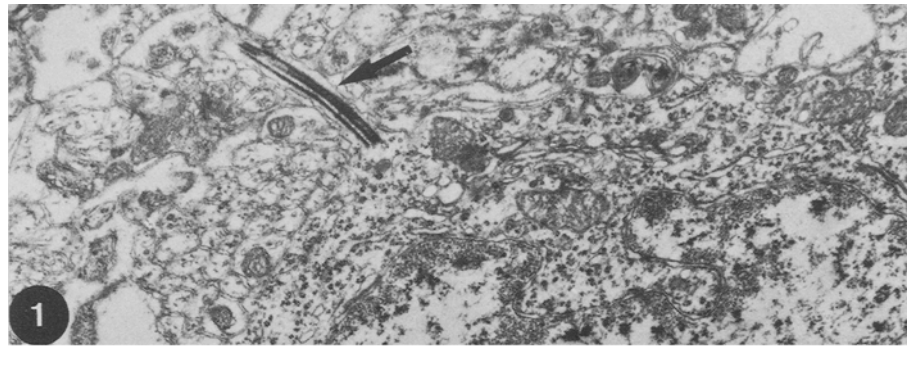

Fig. 1. Cilium cut lengthwise enmeshed in the molecular layer neuropil (arrow). × 14,400.

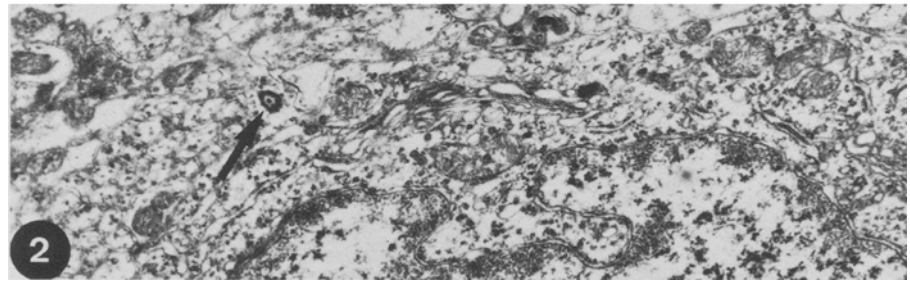

Fig. 2. The same cilium in a different section, cut perpendicularly to its axis (arrow). An 8+1 pattern is recognized. × 14,400.