Effect of Splenectomy on the Growth of Adenovirus 12 Tumors in Hamsters

Splenectomy prior to tumor induction has been shown to inhibit the formation or growth of some transplantable or primary induced tumors[1-10]. This effect has also been observed in the hamster Adenovirus 12 (Adeno 12) tumor system[11]. The in vivo study presented here deals with the effect of splenectomy done before and after tumor cell inoculation on formation and growth of Adeno 12 tumors in hamsters.

Adult Syrian hamsters of a strain kept in closed colony for several years were used. The hamsters showed a high degree of histocompatibility, as indicated by skin grafts performed between randomly selected animals and observed for 300 days. Splenectomy or sham-splenectomy was done by lateral incision under pentobarbital anesthesia (10 mg/100 g body wt.) at times indicated below. The Adeno 12 tumor cell line used for all experiments was induced originally in newborn hamsters by Adenovirus type 12 (Huie strain) and passaged serially in vitro; 2×10^6 cells produced tumors in about 50% and 6×10^6 cells in 100% of adult hamsters.

In a first set of experiments, the effect of splenectomy on tumor incidence, mean latent period and tumor growth was studied in animals inoculated either with a low tumor cell dose (2×10^6 cells/animal) or with a high dose (18×10^6 cells/animal). Splenectomy was done at least 4 weeks before tumor cell inoculation. Control and test animals were inoculated s.c. on the right side with the low or high Adeno 12 tumor cell dose. The tumor growth was determined by measuring 3 perpendicular diameters of the tumor and calculating the geometric mean. The results of these experiments are summarized in Tables I and II. By using the dose of 2×10^6 tumor cells, a marked reduction of the tumor incidence and lengthening of the latent period occurred in splenectomized animals compared with controls. After inoculation of the high dose of tumor cells, only a small difference in the tumor incidence and mean latent period was manifested between splenectomized and control animals (Table I), but a striking difference was observed in the progress of tumor growth (Table II). The tumors of control animals appeared on day 2 and grew progressively until the death of the animals. The tumors of splenectomized animals appeared at about the same time and grew until day 5, but regressed thereafter and did not start to increase until day 12. On day 25 there was still a significant difference in the size of tumors between control and test animals.

In a second set of experiments, hamsters were inoculated s.c. with 12×10^6 Adeno 12 tumor cells and splenectomized or shamsplenectomized either as soon as tumors

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Table I. Influence of splenectomy done before tumor cell inoculation on the tumor incidence and mean latent period

Splenectomy[a]	No. of animals tested	No. of tumor cells inoculated[b]	Incidence[c] of tumors (day 50 after inoculation)	Animals with tumors (%)	Mean latent period[d] (days)
—	16	2×10^6	8/16	50	12
+	11	2×10^6	1/11	9	27
—	12	18×10^6	12/12	100	3
+	9	18×10^6	8/9	89	4

[a] —, No splenectomy; +, splenectomy done at least 4 weeks before tumor cell inoculation. [b] Syngeneic Adeno 12 tumor cells. [c] Number of animals with tumors/number of animals tested. [d] Mean latent period until tumors appeared.

Table II. Influence of splenectomy done before inoculation of 18×10^6 Adeno 12 tumor cells on the course of tumor growth

Days after inoculation of 18×10^6 tumor cells	Mean of the tumor sizes[a] (mm) ($\pm$ standard deviation of the mean) of		Statistic[b]
	8 splenectomized animals	12 notsplenectomized animals	
2	0	0.6 ± 0.3	—
5	4.1 ± 0.4	4.1 ± 0.5	not significant
7	3.2 ± 0.5	6.3 ± 0.7	$p < 0.001$
9	2.2 ± 0.5	9.1 ± 1.0	$p < 0.0005$
12	2.0 ± 0.6	10.1 ± 1.3	$p < 0.0005$
25	12.0 ± 2.8	19.2 ± 2.0	$p < 0.005$

[a] Determined by measuring 3 perpendicular diameters and calculating the geometric mean. [b] Student's t-test.

Table III. Influence of splenectomy and/or BCG treatment done at the time of tumor appearance or during manifest tumor growth on tumor development

Operation	No. of animals tested	BCG treatment at the time of operation	Size[a] of tumors at the time of operation (mm) (mean ± standard deviation of the mean)	No. of animals with tumors on day 50/ No. of animals with tumors at the time of operation	Regression of tumors (%)	Size[a] of tumors on day 25 after operation (mm) (mean ± standard deviation of the mean)	Significance[b]
splenectomy	12	no	2.1 ± 0.3	8/12	33.3	15.5 ± 4.1	$p < 0.05$
sham-spl.	8	no	2.2 ± 0.4	8/8	0	33.8 ± 6.1	
splenectomy	7	no	6.0 ± 0.6	7/7	0	37.1 ± 4.2	not significant
sham-spl.	6	no	7.0 ± 0.6	6/6	0	43.3 ± 3.9	
splenectomy	7	15 mg BCG s.c.	2.6 ± 0.3	5/7	28.5	19.2 ± 5.5	$p < 0.05$
sham-spl.	5	15 mg BCG s.c.	3.2 ± 0.2	5/5	0	37.9 ± 6.0	
splenectomy	7	15 mg BCG s.c.	5.8 ± 0.3	7/7	0	40.5 ± 4.9	not significant
sham-spl.	5	15 mg BCG s.c.	5.8 ± 0.3	5/5	0	37.3 ± 2.8	

[a, b] Explanation see Table II.

were palpable (diameter of 1–4 mm) or when the size of tumors ranged between 5 and 9 mm (geometric mean). The existence of small tumors was ascertained by incision at the side of the tumor and macroscopic examination. Some of these animals received 0.1 ml BCG (15 mg of lyophilized BCG sec Berna, Schweiz. Serum- und Impfinstitut Bern, dissolved in 0.1 ml distilled water) s.c. on the side of the tumor at the time of splenectomy or sham-splenectomy. The results are shown in Table III. Splenectomy done at the time of tumor appearance led to regression of manifest tumors in about 30% of animals and to a significant delay in the growth of tumors in the remaining animals. It is worth noting that we have never observed spontaneous tumor regression in this system before. However, splenectomy had no effect when tumors were already bigger.

BCG treatment known to augment the host's immunological reactivity has been reported to inhibit[12–19], or in some cases to enhance tumor growth[20,21] depending on a number of parameters, e.g. dosage and route of BCG administration, status of tumor immunity of the host and tumor size at the time of treatment[19]. The purpose of BCG application in this work was to study the effect, if at all, of BCG treatment together with splenectomy i.e. inhibition or enhancement of tumors. As seen in Table III, BCG treatment had no effect in addition to splenectomy in these conditions.

The finding that splenectomy done before tumor cell inoculation, or shortly after tumor appearance, inhibited tumor growth, leading even to regression or temporary remission in some cases, points to the important role the spleen may display in promoting tumor growth. With regard to this promoting effect, the spleen probably works by blocking the cellular immune response. Two mechanisms able to block cellular immune reactions against tumor cells are reported, one mediated by antibodies[22,23], possibly antigen-antibody-complexes[24], the other mediated by solubulized antigens[25]. The results obtained suggest the participation of blocking factors produced by the spleen in the tested system. Whether these blocking factors, removable at least partly by splenectomy, are antibodies or whether other more complex mechanisms are involved is now under study.

In conclusion it is reasonable to assume that tumor growth may be promoted by some kind of self-enhancement provided for mainly by the spleen[26].

Zusammenfassung. Die Empfänglichkeit splenektomierter Hamster für Transplantate von Adenovirus-12-Tumorzellen wurde verglichen mit jener von scheinsplenektomierten Kontrolltieren. Splenektomie vor der Inokulation mit Tumorzellen verringerte die Tumorinzidenz oder verzögerte das Tumorwachstum. Splenektomie zum Zeitpunkt des Erscheinens der Tumoren führte immer noch in einem Drittel der Tiere zu Remissionen.

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