Effect of Prior Splenectomy on the Growth of Sarcoma 180 in Normal and Bacillus Calmette-Guérin Infected Mice[1]

Although Sarcoma 180 (S-180) grows progressively in 80–100% of normal mice of certain strains, it does so in face of a demonstrable immune response[2]. Certain agents, such as zymosan and Bacillus Calmette-Guérin (B.C.G.), have been found to render mice appreciably more resistant to S-180 growth, presumably by heightening the immune response or accelerating its development[3–5].

To further explore the elements in the complex immune reaction which follows implantation of a homografted tumor, the possible role of the spleen in the host response to S-180 growth both in normal and B.C.G. infected mice has been investigated. Whereas prior removal of the spleen was not found to abolish the heightened resistance of B.C.G. infected animals to tumor growth, the regression rate of S-180 was increased approximately six-fold in normal Swiss mice previously splenectomized.

Materials and Methods. Mice. Six to eight week old female Swiss Ha/ICR mice, obtained from Millerton Research Farm, were used in these studies. Splenectomy and sham operative procedures were performed under chloral hydrate anesthesia. Animals were challenged with tumors 1 week following operation, except in Experiment E and L in which the interval was 13 days, and 21 days respectively.

Tab. I. Effect of prior splenectomy on S-180 growth in normal and B.C.G. infected mice

Experiment	Non-infected intact	Non-infected sham operated	Non-infected splenectomized	B.C.G. infected intact	B.C.G. infected sham operated	B.C.G. infected splenectomized
A	0/8[a]	0/8	4/8	6/8	8/8	6/6
B	1/8	0/8	6/8	8/9	5/8	6/6
C	1/8		4/7	3/8		7/9
D	1/10		4/10	7/10		4/9
E	1/10	0/10	6/10			
F	2/10			12/15		
H	0/19		14/19			
J	2/8		8/8			
L	0/10		7/10			
Total	8/91 (9%)	0/26 (0%)	53/80 (66%)	36/50 (72%)	13/16 (81%)	23/30 (77%)

[a] Survivors/total. Survivors followed until tumor free.

B.C.G. Infection. Mycobacterium tuberculosis, var. B.C.G., was grown in Dubos tween albumin medium. 7–14 day cultures were harvested by centrifugation, weighed in the wet state, and diluted with fresh medium to 5 mg bacillary mass per ml. 0.2 cm³ (1 mg) of the preparation was injected i.v. into the tail vein to initiate infection. Splenectomy and sham operation were performed 6 days following B.C.G. in Experiments A and B, 13 days in Experiment C and 25 days in Experiment D. B.C.G. infected mice were challenged with S-180 1 week following operation.

Tumor. Sarcoma 180, maintained in this laboratory as a solid tumor, was inoculated as a trocar piece into the right axillary region.

Results. The details of 9 separate experiments and the summary of these results are presented in Table I. The percentage of S-180 regression in 91 control mice was 9%. No animals survived S-180 challenge in the sham operated group in the present study. In contrast, the rate of tumor regression in splenectomized animals was significantly greater than in either controls or sham operated mice in each of 8 experiments. The heightened resistance to progressive S-180 growth in B.C.G. infected mice was also evident and did not appear to be modified by either sham operation or splenectomy prior to tumor challenge. Similar to the behavior of S-180 in B.C.G. infected mice[4], splenectomized non-B.C.G. infected animals supported normal to slightly retarded tumor growth for approximately 3 weeks before the tumor began to diminish in size and finally disappear (Figure Experiment H). Such animals did not demonstrate the characteristic weight loss associated with progressive S-180 growth.

Both splenectomized, and in particular, B.C.G. infected splenectomized mice developed anaemia early in the course of tumor growth. The marked pallor of both ears and paws of splenectomized animals was clearly seen on gross observation of these mice. Modifications in both white and red blood cell counts and haemoglobin values are illustrated in Table II. Each value is the average of data ob-

[1] This work was supported by the American Cancer Society, Inc., New York, N.Y., and The Health Research Council of the City of New York under Contract I-138.

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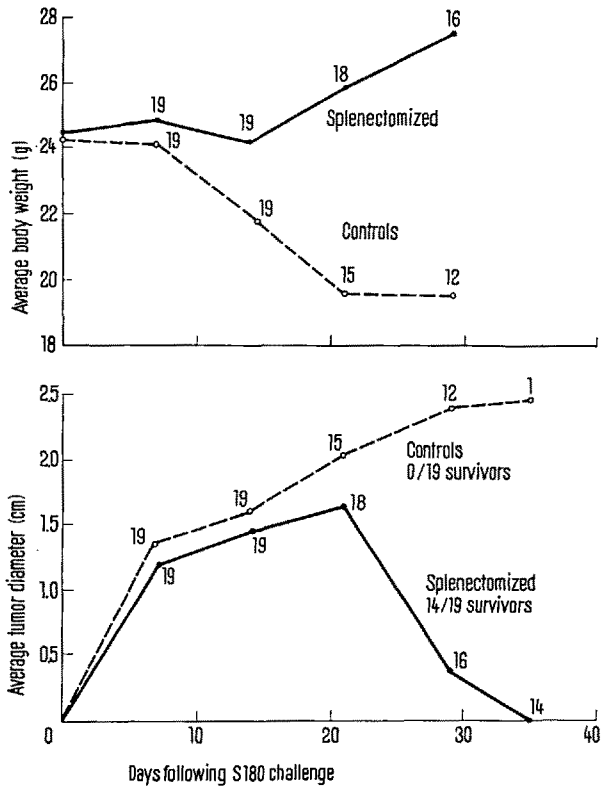
Tab. II. Modifications in the total leukocyte and erythrocyte counts, and haemoglobin values in intact and splenectomized Swiss mice following implantation of S-180

	Days after tumor inoculation	WBC No./mm³ (% Lymphocytes/% Neutrophils) tumor	WBC No./mm³ (% Lymphocytes/% Neutrophils) no tumor	Hb g % tumor	Hb g % no tumor	RBC No./mm³ tumor	RBC No./mm³ no tumor
Intact	7	11.150 (66/29)	7.320 (78/19)	15.2	16.9	9880.000	10282.000
	14	14.600 (38/52)	9.960 (82/13)	16.3	17.0	9684.000	10696.000
	21	22.550 (38/62)	9.760 (87/12)	12.9	16.9	7920.000	9280.000
Splenectomized[a]	7	21.340 (61/33)	17.190 (80/14)	9.6	15.6	6320.000	9244.000
	14	32.890 (52/31)	21.470 (79/16)	8.5	15.2	3884.000	8436.000
	21	39.240 (61/34)	13.190 (84/13)	11.4	16.1	4686.000	8272.000

[a] Splenectomy—1 week prior to S-180 inoculation.



Effect of prior splenectomy on the growth of S-180 and on the body weight of tumor bearing Swiss mice. Figures on chart refer to number of survivors.

tained from 5 mice bled serially from the retroorbital plexus on days 7, 14, and 21 following tumor inoculation. Non-tumor bearing controls are included for comparison. Both haemoglobin values and number of erythrocytes are depressed in splenectomized mice bearing S-180. The total white blood cell count was elevated in tumor bearing and non-tumor bearing splenectomized mice. Leukocytosis and neutrophilia accompanying tumor growth in intact mice was also evident. These haematological alterations in mice bearing S-180 most likely reflect infection by the transmissable agent(s) present in S-180 and a variety of other transplantable tumors[5,6].

Discussion. The increased resistance of splenectomized animals to S-180 challenge is unexpected. Results recorded in the literature might have led one to predict that, if anything, animals lacking spleens would be more susceptible to progressive tumor growth[7]. Part of this increased susceptibility to tumor grafts noted in the past may have been due to factors only indirectly related to absence of the spleen, such as poorer host response to endogenous bacterial infection.

The multiple factors involved in the progressive growth of a homografted tumor in intact animals and regression in splenectomized mice cannot be determined at present. GORER[8] has suggested that the growth of so-called non-specific tumors (S-180, S-37) may be an example of immunological enhancement in untreated animals, the antibody produced by the host acting as the enhancing agent. Thus, the restricted capacity of splenectomized animals to form circulating antibody under certain circumstances[9,10], may result in tumor regression rather than progressive tumor growth. The observations of PREHN[11], who found that prior inoculation of DBA/2 blood by the i.v. route led to enhanced growth of the DBA/2 sarcoma in intact BALB/c mice but relative resistance to tumor growth in splenectomized mice, can be considered as consistent with this interpretation.

Non-immunological factors may, of course, be involved in the increased resistance of splenectomized mice to S-180; among these might be included modifications in the splenectomized host induced by the transmissible agent carried by S-180. It seems likely from current evidence that this agent is identical or closely related to *Eperythrozoon coccoides*, a possibility suggested to us by Dr. P. G. STANSLY, Detroit Institute of Cancer Research. Infection with *E. coccoides* is known to produce anaemia in splenectomized animals, and the degree of anaemia seen in splenectomized tumor-bearing mice in the present experiments probably results from the combined activity of *E. coccoides* and S-180 growth. To eliminate the possible role of *E. coccoides* on the heightened resistance of splenectomized mice to S-180, it will be necessary to suppress or eradicate this organism from S-180 with Terramycin, or to study another homografted tumor not carrying the agent. In one experiment, treatment of mice with 0.5 mg Terramycin subcutaneously for 25 days starting at the time of tumor inoculation did not abolish the higher rate of tumor regression in splenectomized animals.

The fact that splenectomy did not abolish the increased resistance of B.C.G. infected mice to S-180 might suggest that the spleen is not involved in this action of B.C.G. Another possibility, however, is that B.C.G. infection and other agents which modify the structure and function of the spleen promote the regression of S-180 by producing a functional splenectomy. This hypothesis, in the case of B.C.G. at least, seems unlikely since the growth of certain isologous transplantable carcinogen-induced tumors, though inhibited in B.C.G. infected hosts[5], was not altered in splenectomized mice. In addition, B.C.G. appears more efficient as a rule than splenectomy in producing heightened resistance to S-180 challenge. This would not be the case if the effect of B.C.G. infection on S-180 were solely by functional ablation of the spleen.

Zusammenfassung. Die Regression des Sarkomas 180 in Albinomäusen wurde durch vorangehende Splenektomie gefördert. Die gegen Sarkoma 180 erhöhte Resistenz von Mäusen, die mit B.C.G. infiziert worden waren, wird durch die Abwesenheit der Milz nicht beeinflusst.

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