contain the sequence: AAUAAA (A, adenylate; U, uridylate), a feature common to most eukaryotic mRNA's. The 5′ untranslated region is thought to be important for the translation of mRNA molecules. Structural features in this region may be important for ribosome recognition, binding, and translation efficiency. The rat and human preproinsulin mRNA 5′ untranslated regions contain a significant level of homology, when occasional small insertions or deletions (or both) are considered. The predicted secondary structure of the rat mRNA 5′ untranslated region includes a stable hairpin structure that exposes the sequence CCAUCUAGGA (C, cytidylate; G, guanylate) for potential base-pairing with a complementary sequence present in 18*S* ribosomal RNA (*6*, *17*). The corresponding human sequence differs by only one nucleotide and is also contained within a strong potential hairpin structure between the nucleotides at positions 8 and 48 (denoted by asterisks in Fig. 1).

This cloned human preproinsulin cDNA will facilitate the isolation of the human insulin gene, and the information gained from the complete mRNA sequence will permit the distinction between mRNA sequences, intervening sequences, and nucleotide sequences that flank the insulin gene (*18*).

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Role of the Spleen in the Growth of a Murine B Cell Leukemia

Abstract. *A spontaneous B cell leukemia (BCL_1) grew progressively in normal BALB/c mice after injection of tumor cells but did not grow in splenectomized recipients. Despite the absence of progressive tumor growth, residual tumor cells with malignant potential were found in the peripheral blood of the splenectomized animals. Splenectomy performed after injection of tumor cells but before the development of marked leukocytosis also prevented progressive tumor growth and death of the host. Thus the spleen appears to be necessary for progressive proliferation of this lymphocytic leukemia early after passage in vivo.*

We recently described a spontaneous B cell leukemia (BCL_1) of BALB/c mice that expresses B cell surface markers including immunoglobulins M and D, the receptors of the Fc fragment, and I-region antigens (*1*, *2*). The tumor cell line appears unique in its response to stimulation in vitro with the B cell mitogen lipopolysaccharide, since this mitogen stimulates proliferation of and immunoglobulin secretion by the malignant cells (*3*). The tumor cells grow progessively when transferred to normal BALB/c mice and result in their death. Splenomegaly is a consistent manifestation of the disease, and other organs including bone marrow and peripheral blood appear to be involved secondarily (*2*, *4*). Warnke *et al.* (*4*) used autoradiography to demonstrate that there is a pronounced localization of the tumor cells in the spleen early in the course of the disease (*4*). A kinetic study showed that the tumor is manifest in the spleen before large numbers of malignant cells appear in the peripheral blood (*2*). The present study supports and extends these findings by showing that the spleen is required for the progressive growth of the BCL_1 leukemia.

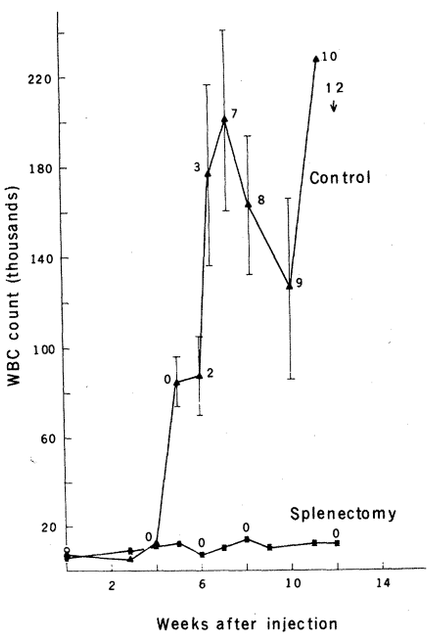

Fig. 1. Growth of BCL_1 tumor cells in splenectomized (N = 24) and normal (N = 12) 4-month-old BALB/c mice. The data are expressed as mean WBC counts ± standard errors. Numbers adjacent to symbols indicate the number of mice dead at that time.

We investigated the role of the spleen in the proliferation of the BCL_1 cells by injecting them into splenectomized mice. The tumor cell line is maintained in our laboratory by passage in unirradiated 2- to 4-month-old BALB/c mice (*5*). To obtain tumor cells for injection, blood was withdrawn from the retro-orbital veins and then diluted in phosphate-buffered saline. The nucleated cells were counted in a hemocytometer. Recipients were splenectomized through an incision in the left upper quadrant under pentobarbital anesthesia. Splenic vessels were tied off with silk, and the incision was closed with metal clips. Twenty-four 4-month-old female BALB/c mice were

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Table 1. Growth of BCL_1 tumor cells in splenectomized mice and after transfer to normal mice. The WBC counts are means ± standard errors.

Time after cell transfer (weeks)	BCL_1 cells given to normal mice* (N = 12)		BCL_1 cells given to splenectomized mice† (N = 24)		WBC's from splenectomized mice given to normal mice‡ (N = 12)	
	WBC count	Deaths	WBC count	Deaths	WBC count	Deaths
0	7,706 ± 344	0	6,885 ± 332	0	8,015 ± 408	0
8	163,750 ± 37,921	4	13,756 ± 522	0	50,700 ± 17,584	1
12		12	14,280 ± 861	0	88,750 ± 17,126	4

†Normal 4-month-old female mice received 10^5 nucleated WBC's from mice with advanced leukemia (WBC count > 200,000). †Splenectomized 4-month-old female recipients received 10^5 nucleated WBC's from mice with advanced leukemia (WBC count > 200,000). ‡Normal mice received 10^5 nucleated WBC's from splenectomized mice 8 weeks after original transfer of tumor cells (mean WBC count of donors, 9700).

splenectomized 3 weeks before the intraperitoneal injection of 1×10^5 nucleated blood cells from tumor-bearing donors with white blood cell (WBC) counts greater than 200,000 per cubic millimeter (the mean normal WBC count is 7,220, as determined in 62 mice of the same sex and age). Twelve littermate female controls also received 1×10^5 nucleated cells from the same tumor cell suspension, but were not splenectomized. Tumor growth was monitored with serial peripheral WBC counts.

Figure 1 shows that progressive leukocytosis developed in the control group but not in the splenectomized group. Twelve weeks after tumor cell injection, all 12 control animals had died from causes related to tumor growth, but all 24 splenectomized mice were alive and had WBC counts less than 20,000. Five months later, the splenectomized animals still showed no evidence of progressive tumor growth. Similar results were obtained after injecting splenectomized mice with either spleen cells or nucleated peripheral blood cells from tumor-bearing mice passaged with spleen cells or with peripheral blood cells (6). When injected with 1×10^5 cells, 12 mice with sham splenectomies developed progressive leukocytosis by 6 weeks, and all died by 16 weeks.

We also investigated the effect of splenectomy after tumor cell injection. Figure 2a compares tumor growth in control mice and in 12 mice splenectomized 4 weeks after tumor cell injection, when the mean WBC count was barely elevated (15,700 ± 2,000). There was marked enlargement of all spleens removed (mean weight, 1.23 g). (The mean normal spleen weight is 0.14 g, as determined in 29 mice of the same sex and age). All 12 of the splenectomized animals showed no evidence of tumor growth 16 weeks after tumor cell injection, whereas 8 of the 12 control animals were dead and the remaining four had developed pronounced leukocytosis. Figure 2b compares tumor growth in control mice and mice splenectomized after the development of marked leukocytosis (mean WBC count, 87,000 ± 20,000). Despite an initial drop in the peripheral WBC count after splenectomy, there was subsequent progressive tumor growth in these mice, and all were dead 16 weeks after tumor cell injection.

Despite the absence of progressive leukocytosis and death in mice splenectomized before tumor cell injection, we found residual tumor cells with malignant potential in these animals. Splenectomized mice injected with tumor cells had significantly higher mean peripheral WBC counts (12,756 ± 522) 8 weeks after injection than splenectomized animals not given tumor cells (6,885 ± 332) (N = 45, $P < .001$, Student's t-test). White blood cells were obtained from the peripheral blood of four different splenectomized mice 8 weeks after the injection of tumor cells, when the mean WBC count was 9,700. Transfer of 1×10^5 WBC's from each splenectomized donor into three normal mice resulted in progressive tumor growth in all 12 recipients (Table 1). The slower tumor growth in these mice compared to the growth rate in control mice (column 1 of Table 1) may be related to the lower number of tumor cells per total number of nucleated cells (that is, a donor with a WBC count > 200,000 might have a greater percentage of tumor cells per 10^5 nucleated peripheral blood cells than a donor with a normal WBC count). The splenectomized mice from which the donor cells were obtained continued to have no evidence of progressive tumor growth (Table 1). Similarly, transfer of nucleated blood cells from a second group of splenectomized mice given tumor cells 12 weeks earlier (without evidence of progressive tumor growth) resulted in progressive growth in normal recipients. In preliminary experiments, we had implanted normal splenic fragments into five splenectomized mice that had been given tumor cells 8 weeks earlier but had not shown progressive leukocytosis. Eight weeks after implantation, two mice were dead from causes related to tumor growth and two had developed marked leukocytosis (WBC count > 50,000).

The results show that the spleen somehow promotes the growth of a transplantable B cell tumor. Although the BCL_1 tumor cells localize in the spleen after being injected intravenously (4), it

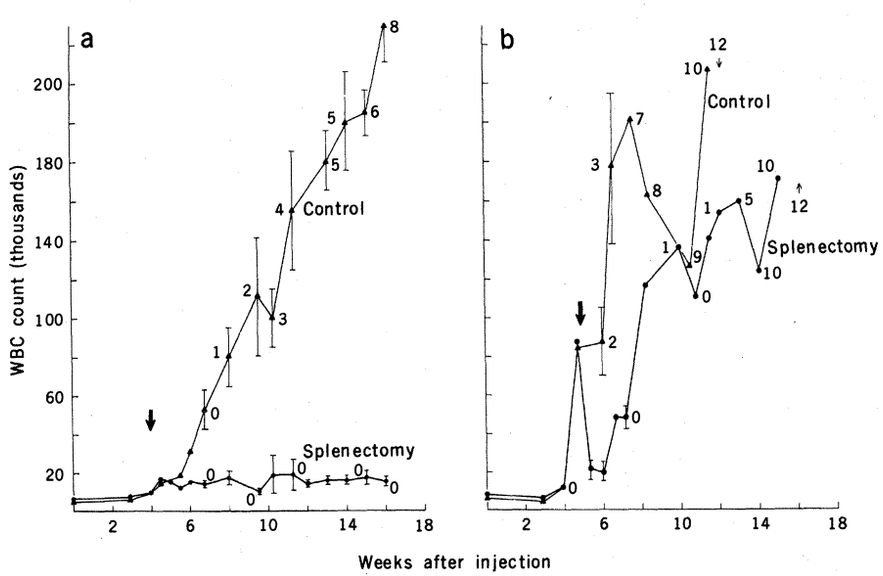

Fig. 2. Growth of BCL_1 tumor cells in mice splenectomized after tumor cell injection and in control mice (N = 12 for each of the four groups). The data are expressed as mean WBC counts ± standard errors. The arrows indicate the time of splenectomy. Numbers adjacent to symbols indicate the number of mice dead at that time. The difference in the rate of tumor cell growth between the control groups in (a) and (b) reflects the usual variability of the results of separate transfer experiments.

is clear that other organs besides the spleen allow for at least minimal proliferation of the tumor. This explains why residual tumor cells could be found for several months in mice splenectomized before tumor cell injection. However, these residual cells did not show progressive malignant growth in the absence of a spleen despite their ability to grow progressively after being transferred to normal mice. In addition, progressive tumor growth in animals splenectomized after the development of marked leukocytosis shows that other tissues can support substantial proliferation during the later phases of tumor dissemination. Thus the spleen may modulate tumor growth in other organs and not just provide an obligatory site for the proliferation of BCL_1 cells.

The mechanism of the interaction between the tumor cells and the spleen cells or their products remains to be elucidated. The spleen may provide a specialized microenvironment for lymphoid tumor proliferation similar to that which supports the proliferation and differentiation of hematopoietic stem cells (*7*). Our demonstration that implantation of normal splenic fragments can initiate progressive tumor growth in splenectomized recipients strengthens this hypothesis. Variations of this experimental protocol (for example, implanting dissociated cells or fragments in Millipore chambers) will help clarify the spleen-tumor interaction. The mechanism by which BCL_1 cells grow progressively after splenectomy late in the course of the disease is also unclear. It is possible that the tumor cells alter their growth requirements, or that the other tissues provide a more "fertile soil" after substantial infiltration by tumor cells.

Other murine B cell tumors also localize in the spleen; the process is related to the differentiation stage and to cell surface characteristics (*8*). However, passage of these other tumor cells to splenectomized mice and subsequent transfer to other mice was not investigated. The modulation of BCL_1 growth by the spleen suggests that the tumor still responds to normal growth or to differentiative signals. Thus this experimental system provides a useful model by which the growth of a tumor similar to chronic lymphocytic leukemia or lymphocytic lymphoma in man can be manipulated by altering normal signals in mice.

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Bone Cancer from Radium: Canine Dose Response Explains Data for Mice and Humans

Abstract. *Analysis of lifetime studies of 243 beagles with skeletal burdens of radium-226 shows that the distribution of bone cancers clusters about a linear function of the logarithms of radiation dose rate to the skeleton and time from exposure until death. Similar relations displaced by species-dependent response ratios also provide satisfactory descriptions of the reported data on deaths from primary bone cancers in people and mice exposed to radium-226. The median cumulative doses (or times) leading to death from bone tumors are 2.9 times larger for dogs than for mice and 3.6 times larger for people than for dogs. These response ratios are well correlated with the normal life expectancies. The cumulative radiation dose required to give significant risk of bone cancer is found to be much less at lower dose rates than at higher rates, but the time required for the tumors to be manifested is longer. At low dose rates, this time exceeds the normal life-span and appears as a practical threshold, which for bone cancer is estimated to occur at an average cumulative radiation dose to the skeleton of about 50 to 110 rads for the three species.*

Knowledge of the effects on people of bone-seeking radionuclides is based primarily on studies of the luminous dial of painters and others who accidentally ingested or were given dosages of radium (*1*). However, the best data relating dosages, radiation dose to bone, and observed effects from exposures to radioactive materials have been obtained from animal studies. In particular, the Department of Energy and its nominal predecessor, the Atomic Energy Commission, have long supported work to develop the beagle as a reliable experimental model (*2*) for radiation dose effects, with the intent of extrapolation to human populations. We used beagle dose-response data for the bone-seeking radionuclide radium-226 to evaluate the relations among radiation dose rate, cumulative dose, time until death, and incidence of bone cancer.

To temporally imitate the medical and occupational exposure of people to ^{226}Ra, 243 purebred beagles of the University of California's Davis colony were administered six graded doses of ^{226}Ra (*3*). Intravenous injections were begun when the beagles were 14 months old and were continued fortnightly until they were 18 months old, so that each dog received eight injections. These dogs and 78 unexposed controls have been under study for the past 18 years.

Skeletal burdens of ^{226}Ra were measured during and after the period of injections by means of whole-body radiation counting of radon daughter gamma-ray emissions with NaI(Tl) scintillation crystal detectors in conjunction with multichannel pulse-height analyzers (*4*). Each measured skeletal activity burden of ^{226}Ra was used to calculate a corresponding dose rate to the skeleton from ^{226}Ra and associated ^{222}Rn and progeny by

$$D(t) = \frac{51.2A(t)[\bar{E}_{Ra} + R(t)\bar{E}_{Rn}]}{m(t)} \quad (1)$$

(*5*), where t is time; $D(t)$ is the dose rate in rads per day; $A(t)$ is activity in microcuries: $\bar{E}_{Ra}$ is 4.86 MeV, or the total energy deposited by decay of ^{226}Ra alpha particles (from recoil nuclei and 4.78- and 4.60-MeV alpha particles); $\bar{E}_{Rn}$ is 20.36 MeV, the average energy deposited by ^{222}Rn with the daughters ^{218}Po, ^{214}Pb, ^{214}Bi, and ^{214}Po in secular equilibrium (of which 19.16 MeV is from alpha particles, 0.34 MeV from recoil nuclei, and 0.86 MeV from beta particles); $R(t)$ is the radon-to-radium activity ratio given by Parks *et al.* (*4*); and $m(t)$ is the mass of the skeleton in grams (*6*). Since the irradiation is primarily alpha, it can be expected to have a quality factor (QF) of about 10 to 20 (*7*). Dose rate values were smoothed numerically with time to yield an average daily dose rate, $\bar{D}$, from the beginning of exposure until death for each dog. For comparison, the unexposed controls were assumed to have re-

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