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THE ROLE OF THE SPLEEN IN TUMOR GROWTH KINETICS OF THE MURINE B CELL LEUKEMIA (BCL_1)[1]

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BCL_1 is a transplantable B cell leukemia resembling human chronic lymphocytic leukemia-lymphoma maintained by cell passage in BALB/c mice. After BCL_1 inoculation (10^7 cells), all mice developed extreme B lymphocytosis in the blood (≤440,000 lymphocytes/mm^3) and marked splenomegaly (50 times normal nucleated cell numbers). BCL_1 infiltrated the spleen before peripheral leukemia was overt (3 days *vs* 28 days, respectively). BCL_1 development in splenectomized mice was characterized by a delayed onset of leukemia (>20,000 cells/mm^3 at 59 *vs* 28 days in intact mice), doubled median survival (102 *vs* 53 days, respectively), and reduced peak level of leukemic counts in the blood (125,000 *vs* 440,000 cells/mm^3). Early splenectomy at different time intervals, ranging between 1 hr to 3 days after BCL_1 inoculation, significantly delayed onset of the disease and prolonged survival, indicating that homing to the spleen occurred as early as 1 hr after inoculation. Splenectomy at 7 days still delayed onset of leukemia but did not affect survival. No significant effect on BCL_1 kinetics was noticed when splenectomy was done on day 21. All splenectomized mice showed significantly lower peripheral blood counts as compared to intact mice (98,000/mm^3 *vs* 440,000/mm^3, respectively). The data show that the spleen plays a major role in the pathogenesis and prognosis of BCL_1.

BCL_1[2] is a B cell leukemia that arose spontaneously in a 24-month-old female BALB/c mouse (1). The disease resembles human chronic lymphocytic leukemia (CLL) by morphologic criteria, cell surface phenotypes, and growth characteristics. Thus, BCL_1 represents the first murine model of chronic B cell leukemia. The tumor has been passaged in syngeneic recipients by intravenous injection of peripheral blood cells. Inoculation of BALB/c mice with as few as 10 BCL_1 cells results in leukemia in 100% of the recipients. The cells in the peripheral blood have the normal morphologic appearance of medium size lymphocytes and reach cell counts of up to 600,000/mm^3 (1, 2). The tumor cells bear H-2^d alloantigens, IgM, small amounts of IgD, Fc receptors, and Ia antigens encoded by the E subregion (1-4). The cells are monoclonal as determined by the λ-light chain type and a single idiotype (3-5). The most striking feature of the tumor is extreme splenomegaly, with cell counts reaching 10^9 to 10^{10} nucleated cells per spleen (50 times normal) (1-3). Histopathologic studies have demonstrated infiltration of organs including bone marrow, peripheral lymph nodes, lungs, kidneys, and liver (6). A variety of experiments indicates that BCL_1 grows initially in the spleen and then spreads to the peripheral blood (6, 7). These studies further suggest that BCL_1 cells may undergo a differentiating event in the spleen, analogous to normal B cells. To test this hypothesis and to examine the role of the spleen in tumor growth and therapy, we have splenectomized mice before and after BCL_1 inoculation.

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[1] Supported in part by the National Institutes of Health Grant AI 15387 and by a grant from the United States-Israel Binational Science Foundation.
[2] Abbreviations used in this paper: BCL_1, B cell leukemia; CLL, chronic lymphocytic leukemia.

MATERIALS AND METHODS

Experimental mice. Inbred 5-month-old BALB/c female mice were used for inoculation and maintenance of BCL_1 in all experiments. Each experimental group consisted of six mice that were monitored individually for all parameters tested.

Source of BCL_1 cells. The BCL_1 was maintained *in vivo* by intravenous passage of 10^6 to 10^7 peripheral blood cells in BALB/c mice (1). BCL_1 cells were obtained by collection of blood from the retro-orbital veins of BCL_1-bearing BALB/c mice into heparinized tubes. The blood was diluted with saline to the appropriate cell concentration.

Induction and monitoring of BCL_1. Leukemia was induced by intravenous injection of 10^7 BCL_1 cells per mouse in all experiments. BCL_1 was monitored by repeated peripheral blood counts on a weekly basis. Blood was obtained from the retro-orbital veins and the white blood count was determined by diluting the blood in 2% acetic acid using a hemacytometer. Mice were considered leukemic when peripheral lymphocyte counts exceeded 20,000/mm^3.

Splenectomy. Splenectomy was performed in some experiments 14 days before, and in others at different time intervals following inoculation with BCL_1. The spleen was exposed through a left abdominal incision and the pedicle was clamped and cauterized. The incision was closed with metal clips.

Determination of leukemic involvement of the spleen. Splenic infiltration was evaluated by comparing the whole organ weight to that of an intact age-matched control and by counting the total cell content after teasing into a single cell suspension using a metal mesh.

Determination of leukemic involvement of the liver and lymphoid organs. Age and sex matched intact and splenectomized mice were sacrificed 95 days after intravenous injection of 10^7 BCL_1 cells. BCL_1 involvement of different organs was evaluated by comparing the weight and cell content of the organs in these two groups and normal controls. The four brachial

lymph nodes, the two inguinal lymph nodes, the mesenteric nodes, and the spleen were examined separately. The cell content was evaluated by teasing the lymphoid organs through a nylon mesh and counting the total cells recovered. The liver was excized and weighed in all groups.

Statistical analysis. Comparisons between leukemia preincubation periods and survival of BCL_1-inoculated mice subsequent to different experimental procedures were done using the Kolmogorov-Smirnov two-sample test (8).

RESULTS

Development of leukemia after BCL_1 inoculation. BCL_1 inoculation in all the experiments reported herewith consisted of intravenous injection of 10^7 peripheral blood lymphocytes obtained from leukemic mice. All recipients developed consistent leukemia (>20,000 lymphocytes/mm^3) with peripheral blood lymphocyte counts of up to 450,000/mm^3 (Fig. 1). After injection with BCL_1, significant leukemic lymphocytosis occurred between 21 and 42 days, with a median of 28 days (Figs. 1 and 2).

BCL_1 development was accompanied by extreme splenomegaly (Tables I and II). Less striking was the leukemic infiltration in other lymphoid and nonlymphoid organs such as the liver (Table I). There appeared to be no significant increase in the weight and cell number of peripheral lymph nodes (brachial and inguinal) at 94 days after BCL_1 inoculation, except for the mesenteric lymph node chain (Table I). Infiltration into the liver was also quite extensive since the size of that organ increased by more than 2-fold (Table I).

Splenic involvement in BCL_1. A significant increase in spleen weight and cell number was already noticed 3 days after BCL_1 inoculation (Table II). Spleen weight increased 10-fold at 21 days and 20-fold at 94 days after BCL_1 inoculation (Table II).

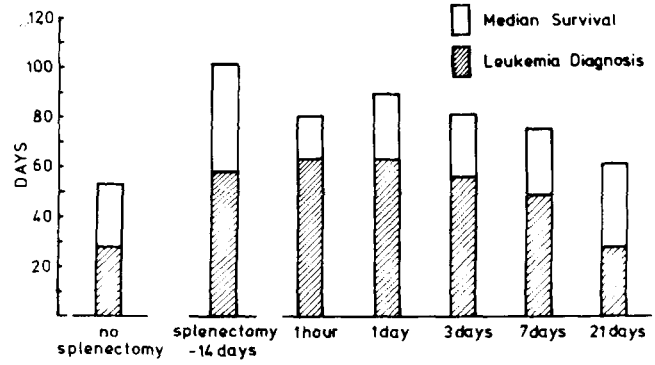

Figure 2. Median time interval of BCL_1 onset (>20,000 lymphocytes/mm^3 in 50% mice) and median survival of BALB/c mice inoculated with 10^7 BCL_1 cells, 14 days after splenectomy or at different time intervals before splenectomy. Each group consisted of six mice. The delay in the onset of leukemia (>20,000 lymphocytes/mm^3) as compared to nonsplenectomized controls was significant only in the groups of mice that underwent splenectomy 1 hr ($p = 0.01$), 1 day ($p = 0.01$), 3 days ($p = 0.05$) and 7 days ($p = 0.05$) after BCL_1 inoculation. Increased survival of splenectomized leukemic mice as compared to nonsplenectomized leukemic controls was significant only in the groups of mice that underwent splenectomy 1 hr ($p = 0.05$), 1 day ($p = 0.05$) and 3 days ($p = 0.05$) after BCL_1 inoculation.

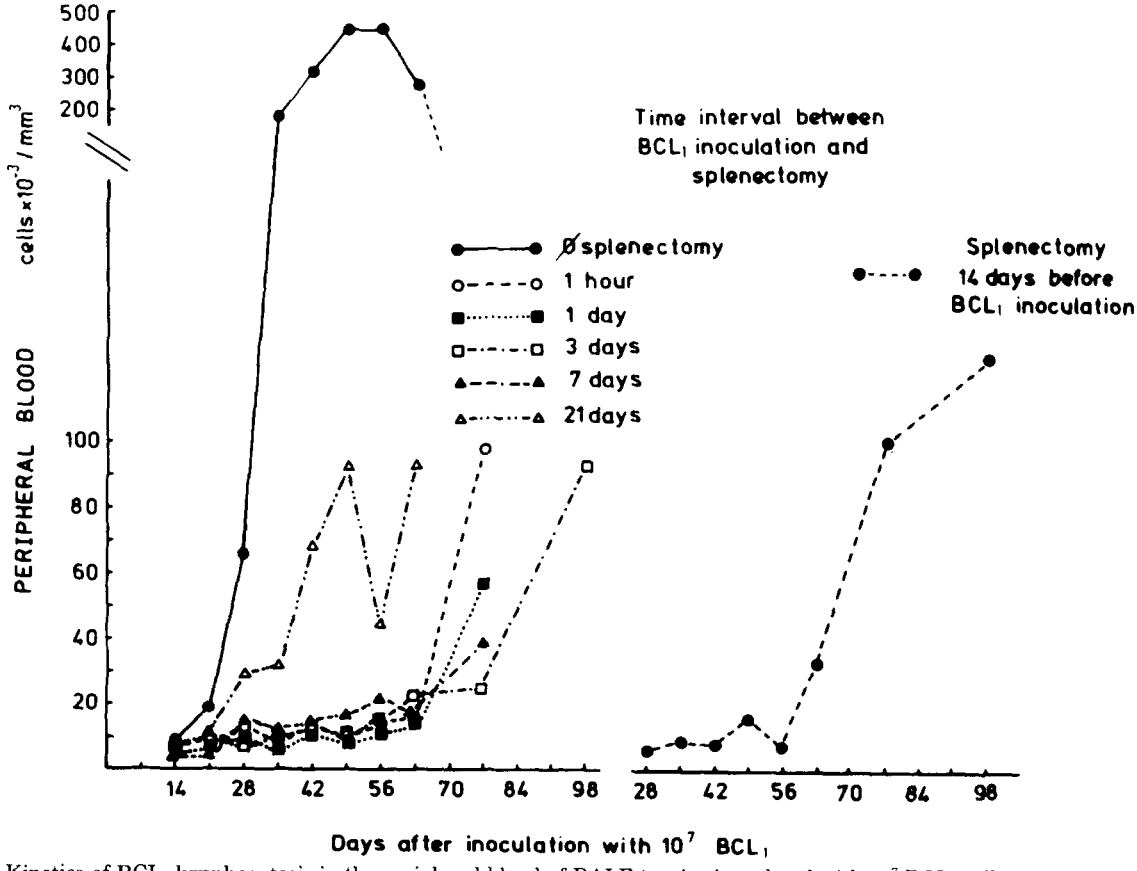

Figure 1. Kinetics of BCL_1 lymphocytosis in the peripheral blood of BALB/c mice inoculated with 10^7 BCL_1 cells 14 days after splenectomy or at different time intervals before splenectomy. The values represent a mean of individual blood samples obtained on a weekly basis. Each group consisted of six mice.

TABLE I

Weight and cell number of lymphoid organs and liver of normal and splenectomized BALB/c mice 94 days after i.v. inoculation with 10^7 BCL_1 cells

	Brachial LN[a]		Inguinal LN		Mesenteric LN (Pool)		Liver Weight	Spleen Weight
	Mean weight	Mean cell no.	Mean weight	Mean cell no.	Weight	Cell no.		
	gm		*gm*				*gm*	
Normal controls	0.0018 ± 0.0005[b]	0.72×10^6	0.0021 ± 0.0003[b]	1.4×10^6	0.0500 ± 0.0010[b]	43×10^6	1.2900 ± 0.0400[b]	0.1400 ± 0.0100[b]
Intact mice injected with BCL_1	0.0013	1.81×10^6	0.0022	1.1×10^6	0.2170	266×10^6	4.0590	2.5571
Splenectomy 14 days before inoculation	0.0550	40×10^6	0.0420	18×10^6	0.2690	138×10^6	2.0760	

[a] LN, lymph node.
[b] Mean ± S.D.

TABLE II

Spleen weight and cell content at different time intervals after i.v. injection with 10^7 BCL_1 cells

	Time Interval between BCL_1 Inoculation and Splenectomy			
	1 Hr	3 Days	7 Days	21 Days
Mean spleen weight ± S.D. (gm)	0.140 ± 0.010	0.189 ± 0.011	0.305 ± 0.033	1.070 ± 0.140
Mean no. of cells/spleen ± S.D. × 10^6	173 ± 30	206 ± 29	311 ± 50	1,500 ± 450

Statistical analysis of the kinetics of cell multiplication in the spleen revealed a doubling time of 5.2 days. Splenic infiltration with BCL_1 preceded leukemic lymphocytosis in the blood, as will be clearly demonstrated when comparing cell kinetics in Table II and Figure 1.

Survival of mice inoculated with BCL_1. Death occurred between 55 and 149 days after BCL_1 inoculation in all mice, with a median survival of 53 days (Fig. 2).

BCL_1 kinetics in splenectomized mice. BCL_1 kinetics and survival were studied in six mice that underwent splenectomy 14 days before BCL_1 inoculation and in six age-matched intact controls. Leukemia developed in 50% of intact mice at 20 days and in 50% of spleenless mice at 63 days after inoculation ($p = 0.01$). The peak values of peripheral blood counts were higher in intact mice as compared to splenectomized animals (450,000 and 125,000/mm^3, respectively) (Fig. 1).

The spleen played a major role in the survival of leukemic mice. The median mortality was 53 days (range 45 to 149 days) in intact mice and 102 days (range 96 to 112 days) in splenectomized mice ($p = 0.05$) (Fig. 2).

The presence of the spleen enhanced and accelerated BCL_1 development as well as aggravated prognosis.

Kinetics of leukemia after splenectomy at different time intervals of BCL_1 inoculation. Early removal of the spleen—1 hr to 3 days after BCL_1 inoculation—significantly delayed the onset of leukemia and prolonged survival (Figs. 1 and 2) indicating that most leukemic cells homed to the spleen at an early stage. Splenectomy at day 7 was still effective in delaying BCL_1 diagnosis, but did not prolong the survival to a significant extent. Neither the appearance of leukemia nor survival were affected by late splenectomy at day 21 after BCL_1 inoculation (Figs. 1 and 2).

BCL_1 development in the absence of the spleen was characterized by a significant enlargement of the peripheral lymph nodes as compared to intact BCL_1 mice or normal controls, including an increase of the weight and cell content of the brachial (40×) and inguinal (20×) lymph nodes (Table I).

Interestingly, the degree of leukemic infiltration in the blood was significantly lower in all splenectomized mice, with mean blood levels of up to 98,000/mm^3 as compared to 450,000/mm^3 in mice with intact spleens (Fig. 1).

It can be concluded that early splenectomy of BCL_1-bearing mice increased the pre-leukemic course, diminished the degree of leukemic infiltration in the blood, and prolonged survival. BCL_1 was never totally eliminated by splenectomy at any time interval after inoculation.

DISCUSSION

Morphologically, BCL_1 cells resemble mature lymphocytes, whereas, by cell surface markers the characteristic tumor cells can be defined as early B cells (1-3). Histologically, the lymphomatous spleen has the appearance of well-differentiated lymphoma (6). Growth kinetics indicate that the spleen is the first and main target organ of the BCL_1 tumor (6, 7). A significant increase in spleen size and cell content was demonstrated as early as 3 days post-BCL_1 inoculation, whereas leukemia was diagnosed in the peripheral blood only on day 28. The predilection of the tumor cells for the spleen offered the opportunity of studying the role of this organ in the biology of the BCL_1 disease. Since most BCL_1 cells homed to the spleen at a very early stage after inoculation, it was of interest to determine whether BCL_1 could be cured or ameliorated by splenectomy. The data indicated that BCL_1 could not be eradicated by splenectomy alone at any time interval between 1 hr and 21 days post-inoculation. Nevertheless, elimination of a large tumor load was possible by early removal of the spleen, best demonstrated by a significant delay in the appearance of overt leukemia and prolongation of survival. Late splenectomy had no significant effect on BCL_1 kinetics. The circulating cell number in splenectomized mice was never as high as that observed in intact animals, suggesting an augmentative role of the spleen in tumor development. The latter issue reveals some theoretical as well as therapeutic implications regarding the possible curative role of splenectomy in well-differentiated lymphoma before and after the occurrence of CLL. Splenectomy has been suggested as a promising procedure in the treatment of the prolymphocytic variant of CLL (9). The present findings, using the BCL_1 animal model that bears many similarities to the prolymphocytic subset of CLL, support this therapeutic approach.

Variants of B cell leukemia-lymphoma disorders have been known for many years in man. The relationship between the

presence of B cell type tumors in the spleen and the appearance of overt leukemia may shed light on the interrelationship between human prolymphocytic leukemia, well-differentiated lymphoma, lymphosarcoma-cell leukemia, and CLL. Splenomegalic-type CLL without obvious lymphadenopathy was described in about 12% of CLL cases in one series (10), and others have defined the unique variant of CLL with splenomegaly and extreme B lymphocytosis as prolympholytic leukemia (9). Marrow involvement and leukemic peripheral blood picture may also complicate the terminal phase of lymphosarcoma, the so-called lymphosarcoma-cell leukemia, occurring in up to 10% of patients (11–14). The BCL_1 animal model provides a useful experimental tool in sorting out the complexity of the immunology and immunopathology associated with B cell biology and neoplasia. It also affords the study of the lymphocytic leukemia-lymphoma entity in relation to clinically relevant problems such as ontogeny, pathogenesis, tissue distribution, and therapy.

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