MEDICAL GRAND ROUNDS

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CHRONIC LYMPHOCYTIC LEUKEMIA

AND RELATED

LYMPHOPROLIFERATIVE STATES

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I. REPRESENTATIVE CLINICAL CASES:

1. M.B.: At age 52 (1957) this woman, involved in health care delivery, was seen for her routine annual physical examination. She had no clinical complaints and her physical examination was completely normal. An absolute lymphocytosis was noted on examination of her peripheral blood (see below). No other clinical or laboratory abnormalities were noted. Subsequent to that initial evaluation she had a persistent lymphocytosis and her peripheral hematologic values can be seen in the table below. Over the past 20 years she has been followed serially by her family internist and seen in recent years in consultation here at the Medical School. During this time she has been completely asymptomatic and has had no significant changes in her physical findings. In particular, she had neither lymph node enlargement nor hepatosplenomegaly. During the 1960's bone marrow aspiration was performed and demonstrated significant lymphocytic infiltration of the marrow providing confirmation of the diagnosis of chronic lymphocytic leukemia.

In addition to the laboratory findings noted below she has had normal serum and urine proteins, a negative Coombs' test, and a normal profile by SMA-12 evaluation.

She has continued in robust health to the present with no change in physical findings. Subsequent to her retirement from active employment she became a volunteer health care worker and has continued to be very active in this role.

Laboratory Data:

	WBC	% Lymph	Absolute Lymphocyte	Hb/Hct	Platelets
6/'57	14,800	56	8,288	13.5/39	Adq.
8/'60	9,500	52	4,940	13/39	-
12/'64	12,100	62	7,502	14/41	277,000
6/'68	43,000	75	32,250	12.5/38	205,000
10/'68	62,000	85	52,700	13.7/40	250,000
1/'69	55,000	80	44,000	13.5/39	240,000
8/'69	88,000	86	75,680	14/40	202,000
7/'77	59,000	85	50,150	14/40	225,000
7/'78	48,000	85	40,800	14/40	245,000

2. Dr. C.: This 40-year old physician was first seen in 1968, having been referred by his family pathologist with a diagnosis of acute leukemia. He apparently had been asymptomatic but had noted a small node in his right cervical area. While awaiting his third surgical case for the day his associate elected to excise the node. Frozen section report was "diagnostic of acute leukemia". Peripheral hematologic examination

was then performed and his white count noted to be 300,000/mm^3, "all of which are myeloblasts". A diagnosis of acute leukemia was made and the patient was referred for evaluation and therapy.

When seen on the following morning (15 April 1968) his only identifiable symptoms were a 10-lb. weight loss in the previous few months (but while trying to lose weight by caloric restriction) and occasional night sweats. Initial evaluation revealed him to be well developed and slightly obese. He had generalized enlargement of his lymph nodes with multiple nodes (2-3 cm in size) in the anterior and posterior cervical chains and supraclavicular areas. Multiple axillary nodes were present bilaterally, some of which were 4 cm in diameter. His liver was palpable 4 cm beneath the right costal margin with an over-all width of 18 cm, and his spleen was palpable 15 cm beneath the left costal margin. Initial hematologic evaluation confirmed that the white blood count was 310,000, of which 99% were small, mature lymphocytes. His hemoglobin was 13.8 gms; hematocrit 43 vols%; and his platelets 175,000. Other laboratory studies revealed a borderline gamma globulin in the range of 0.9-1.0 gm/dl and a serum uric acid of 12 mg/dl. Evaluation of the morphologic material confirmed the diagnosis of chronic lymphocytic leukemia.

The patient was started on prednisone and over the next 6 weeks had only trivial change in his peripheral hematologic values, but he had complete disappearance of all of his peripheral lymphadenopathy and a decrease in his spleen size to 9 cm beneath the costal margin. Possible therapeutic strategies were discussed with the patient and as the prednisone was tapered to 30 mg/day high dose chlorambucil was added to his program on 5 June 1968. Over the next 5 months he had a progressive decline in his peripheral white count from the 200,000 to 250,000 range to approximately 50,000/µl. His hemoglobin ranged between 12 and 13 gms; and platelets in the range of 100-150,000.

It is noteworthy that in spite of decline in his peripheral circulating white count spleen size actually increased; thus, by November 1968 his spleen was once again felt 15 cm beneath the left costal margin and his peripheral lymphadenopathy recurred and were up to 2 cm in the axillary and supraclavicular regions. Continued therapy led to progressive thrombocytopenia with little change in circulating lymphocyte levels. In light of these changes and the limitations posed by the thrombocytopenia the chlorambucil was discontinued. Since the strategy continued to be extended reduction in mass disease cyclophosphamide was instituted. Over the next 4 months he had a progressive decline in his white count to the range of 3-5,000 and a progressive emergence of polymorphonuclear leukocytes so that his peripheral differential count returned to a pattern that was within normal limits. His hemoglobin rose to 15 gm/dl, hematocrit 45 vols% and his platelets 452,000/µl. Over the next few months there was a slow and progressive disappearance of all peripheral lymphadenopathy and a disappearance of the previously identifiable hepatosplenomegaly. By the spring of 1969 he had achieved a <u>complete</u> hematologic remission with no evidence of abnormalities identifiable either on physical examination or laboratory examination.

The complete remission was maintained for approximately 4 years. In the summer of 1973 he had an increase in his peripheral circulating lymphocytes as well as the development of some peripheral lymph nodes and a palpable spleen. In late summer of that year he had a severe episode of herpes zoster that was treated with Ara-A. Subsequent to the episode of zoster he developed an abscess in his left groin due to *Pseudomonas*. *Pseudomonas* cellulitis and septicemia ensued and the patient died in late 1973 secondary to the septic episode.

3. Ms. M.: This 62-year old woman was first seen in the summer of 1974 after splenomegaly had been identified by her family physician. Her clinical story was quite unremarkable except for a history of severe joint pain and swelling that had led to a longstanding diagnosis of rheumatoid arthritis and extensive use of Butazolidin in the past. When first seen she was asymptomatic except for pains in the small joints of her hands and feet. Initial physical examination revealed no significant skin lesions or lymphadenopathy. Her spleen was palpable 8 cm beneath the left costal margin in the midclavicular line. Only slight evidence of synovial thickening was present in the proximal MT joints.

Laboratory studies revealed a hemoglobin of 15 gms with a hematocrit of 42 vols%. Her initial white count was 12,600 with 82% lymphocytes with large, hyperchromatic nuclei and prominent nucleoli evidenced on supravital stains. Her platelets were 190,000. The remainder of her laboratory findings were within normal limits and bone marrow aspiration and biopsy revealed that approximately 90% of the marrow was replaced with cells of the same architectural type noted in the peripheral blood. Surface marker studies demonstrated bright fluorescence with evidence of both IgM and IgD markers on the cells.

The patient remained asymptomatic and in a stable clinical state over the next several months. In early 1975 she developed severe constitutional symptoms characterized by drenching night sweats and daily fevers ranging to 104°. Over the next several months she was extensively evaluated at three separate medical centers in a search for a potential mechanism (beyond her primary disease) for these constitutional findings. No specific infectious mechanism was identified and the patient was treated at various centers with multi-agent therapy on the empirical consideration that an opportunistic infection was the cause of the symptoms. These attempts failed to result in a reduction in the severity of her constitutional symptoms.

A trial of "thymic" (mediastinal) radiation resulted in a modest decline in her white count (range 8-12,000 with 75% lymphocytes), a decrease in her splenomegaly (to 4 cm beneath the costal margin) and a disappearance of her fever and related symptoms for approximately 4 months. The recurrence of symptoms and the advent of progressive splenomegaly with a decline in hemoglobin to 10 gms/dl and an increase in her white count to

the 25-30,000 range (with 85% of the cells as described) led to abdominal exploration and elective splenectomy. Following her splenectomy her symptom complex again markedly decreased. After a few months of respite the constitutional symptoms recurred and were only slightly affected by serial courses of multi-agent chemotherapy administered at a variety of medical institutions in the United States. Although her symptoms were severe she was a stoic lady and functioned reasonably normally over the subsequent 4 years in spite of her symptoms and the attempts at therapeutic intervention.

II. DIAGNOSTIC CRITERIA - CHRONIC LYMPHOCYTIC LEUKEMIA (C.L.L.):

A. General Criteria of Diagnosis:

Review of the literature is noteworthy in the lack of specific criteria for the diagnosis of chronic lymphocytic leukemia. In general most texts indicate that the diagnosis is easily made and a loose requirement for 10,000 or 15,000 lymphocytes/µl is generally applied. Bone marrow material has been considered "optional"; the "appropriate" diagnostic values have been reported to consist of a lymphocytosis in excess of 25 to 50% of the non-erythroid marrow nucleated cells.

Recent clearer definitions of malignant lymphoma and interest in B cell neoplasms have focused attention on clearer criteria for the diagnosis of C.L.L. Rappaport has proposed that adults with greater than 4,000 lymphocytes per microliter can be labeled C.L.L. (1). This level was based on the Stanford malignant lymphoma experience; nevertheless, the actual original expression was that: "Relatively arbitrary criteria were used to distinguish patients with C.L.L. from patients with lymphoma; that is, 1.) lymph node histology of the diffuse, lymphocytic well differentiated type; 2.) peripheral lymphocytosis of 4,000 or more/µl; and 3.) a bone marrow diffusely infiltrated with well differentiated lymphocytes.... no patient referred to the Stanford unit fulfilled these criteria" (2).

More rational criteria can be generated from reviews of the extended studies of "normal" populations (3, 4). In general, the data from such studies have provided "reference ranges" of values (5). It is clear that lymphocyte numbers in adults have a non-Caucasian distribution and that the upper range of absolute lymphocyte values in normal populations is approximately 4,500/µl. From these studies criteria can be posed for values in the adult [consistent with] that are "diagnostic of C.L.L.:

- Peripheral blood lymphocytosis in excess of 4,800 per µl.
- Bone marrow lymphocytosis in excess of 25% of the non-erythroid nucleated cell mass, when these are not associated with a lymphoid follicle and general marrow cellularity is not decreased.

At marginal absolute lymphocyte counts in the peripheral blood, it is assumed that counts on two or more occasions at least 24 hours apart are obtained because some degree of cyclic variation occurs in lymphocyte traffic, albeit considerably less than that seen in granulocyte members (6).

The term C.L.L. as used in texts of medicine encompasses several separate clinical patterns. The current discussion will focus on:

1.) Chronic lymphocytic leukemia - B cell type
2.) Chronic lymphocytic leukemia - T cell type
3.) Prolymphocytic leukemia
4.) Lymphosarcoma leukemia (so-called "spill-over" leukemia)

Other leukemic lymphoproliferative lesions include acute lymphocytic leukemia and its variants, the leukemic phase of lymphoblastic (T cell) lymphoma, leukemic reticuloendotheliosis (LRE), and Sézary syndrome; these separate topics will not be included in the present discussion.

B. "Leukemoid" Lymphocytic Reactions:

An adult counterpart of the common pediatric finding of a "lymphocytic leukemoid reaction" is so rare that its potential is often disregarded. Although such responses are uncommon in adults, they have been described especially in the presence of chronic infectious granulomatous disease (7), and with other solid tumors (8, 9, 10). Bichel (10), for instance, described a patient (one of three similar cases) found to have a WBC of 250,000/µl of which 88% were lymphocytes at the time of an initial diagnosis of adenocarcinoma of the stomach. The patient lived 6 years post resection and his peripheral hematologic values paralleled the clinical activity of the gastric neoplasm; at autopsy no evidence of a lymphocytic neoplasm could be identified.

III. THE "NATURAL" HISTORY OF C.L.L.:

In the 100 years since Virchow's application of the term "leukemia" and his characterization that the lymphoid mass was the source for the lymphocytic type (11), we have made but trivial progress.

An oft quoted description by Gunz in 1974 (12):

> "A typical patient with chronic lymphocytic leukemia (C.L.L.) is a man, aged 55, who recently noted an (or multiple) enlarged non-tender cervical lymph node; he is otherwise quite well. Examination reveals generalized lymphadenopathy and splenomegaly. Evaluation

> of his peripheral blood reveals a white blood count of 60,000/µl and that 96% of the cells are small (mature) lymphocytes. Red cell and platelet values are normal. The bone marrow is diffusely infiltrated with the same cells seen in the peripheral blood. Biopsy of the lymph node reveals a diffuse well differentiated 'lymphoma'."

This description differs little from the formidable clinical review by Minot and Isaacs 50 years ago (13) of 98 carefully studied cases of C.L.L. They defined C.L.L. as:

1.) A disease of adulthood (>age 40) with peak incidence 45-65 years.

2.) Affects men about 3 times more frequently than women.

3.) The insidious nature of the onset of disease was highlighted by evidence of a delay of 1.4 years between the first (retrospective) symptom and the establishment of a diagnosis. Indeed, the average patient did not seek medical attention for his symptoms for approximately 6 months.

4.) The average duration of the disease (expressed as the length of life after its onset) was 3.5 years. Presentation with C.L.L. below the age of 40 carried a somewhat shorter survival likelihood.

5.) Therapeutic effectiveness (with radiation) was difficult to judge. Clearly it did provide a "detectable effect on the duration of life", since the treated patients had a survival of 3.45 years (compared to the "control" group of 3.5 years). Even more critical is the advice regarding caution in interpreting therapeutic adventures since:

a. "In judging the effects of therapy one must recall that spontaneous remissions occur in C.L.L." [actually in about 5% of their cases].

b. "Remarkable decreases in lymph node size can occur in the absence of therapy, although often only temporary." [in about 10% of their cases].

c. "WBC and spleen size also may fluctuate in the absence of therapy.

d. "Change in the height of the WBC is not a valid indicator of clinical benefit, since symptomatic improvement was not necessarily associated with changes in peripheral counts." [They reduced the WBC to "normal" in 60% of their cases by means of radiation, but no selective survival advantage could be demonstrated in that group.]

e. "The hemoglobin level and the platelet numbers serve as important criteria to adjudge the patient's condition."

f. "Spleen size varies considerably during the course of the disease but bears no relationship to the state of the patient, the condition of his blood or the future course of his disease, nor do these different factors necessarily fluctuate together." [This may not be completely correct in a selected sub-group where "hypersplenism" may be a factor in the patient's clinical state.]

Helping set the "Oncologic-Style" of unbridled therapeutic optimism, Minot and Isaacs concluded: "The knowledge of today...will permit still greater benefits from therapy in C.L.L. than in the past 10 years!"

In spite of our broadened understanding of certain clinical issues in C.L.L. (e.g. immunoglobulin alterations and warm-antibody mediated hemolytic anemia), the advent of expanded therapeutic approaches (e.g. radiation, chemotherapy) and the immunologic explosion (e.g. antilymphocyte/antithymocyte globulin) survival trends for C.L.L. have changed but little (14).

IV. CLINICAL SPECTRUM OF C.L.L.:

Although virtually every clinical review of C.L.L. describes the clinical course as variable - "It is more variable in clinical features and natural history than any other form of leukemia.." (12) - the clinical manifestations can reasonably be projected from the alterations in function and/or structure of the lymphoid mass (15, 16, 17).

TABLE I

CLINICAL FINDINGS IN C.L.L.

1. Symptoms and Findings Related to Altered Lymphocyte Function:

Common:

- Autoimmune hemolytic anemia (i.e. antibody mediated hemolytic anemia)
- Immunologic thrombocytopenic purpura (i.e. antibody mediated thrombocytopenia)
- Immunoglobulin alterations (i.e. hypogammaglobulinemia [18, 19]; cryoglobulinemia [20, 21], monoclonal gammopathy [22])- [Every paraprotein class has been associated with C.L.L.]
- "Opportunistic" infections

Uncommon:

- Vasculitis (primarily of skin); thyroiditis; Mikulicz-Sjögren syndrome

2. Symptoms and Findings Related to "Infiltrative" Lesions:

Common:

- Hematopoietic changes (i.e. anemia, thrombocytopenia)
- Skin lesions (7-10% of cases) (exfoliative erythroderm, nodules, etc.)
- Skeletal alterations (bone pain)

Uncommon:

- Pleural effusions
- Orbital involvement
- Meningeal involvement
- Diffuse interstitial pulmonary infiltration
- Hepatic dysfunction

TABLE I (Continued)

3. Symptoms and Findings Related to Lymphoid-Mass Change:

Common:

- Obstruction of G.U. system
- Spinal cord compression
- Hypersplenism (with -cytopenias)

Uncommon:

- Intestinal obstruction
- Superior vena cava syndrome
- Upper airway obstruction
- Auditory nerve compression (tinnitus → deafness)
- Splenic rupture

4. Other Potential Clinical Features:

- Generalized vaccinia or vaccinia gangrenosa (following smallpox vaccination)
- Multifocal leukoencephalopathy (diffuse demyelination syndrome)
- Acropachy (clubbing with symmetrical destruction of terminal phalanges and edema of the overlying skin: allegedly "pathognomonic for C.L.L." [23])
- Phenomenon of the second primary neoplasm:
  Second primary neoplasms occur in patients with C.L.L. more frequently than can be accounted for by chance alone; it is second only to skin as a circumstance where the "second primary" phenomenon occurs (24)
- Acquired von Willebrand's syndrome (bleeding responsive to cryoprecipitate or leukemic cytoreduction)

Another approach to the characterization of those clinical manifestations in C.L.L. that can be considered significant is to consider the causes of death in patients with chronic lymphocytic leukemia:

TABLE II

CAUSES OF DEATH IN C.L.L. (25)

		Percent
Death related to C.L.L.:		54%
- due to infection	46%	
- due to thrombocytopenia	4%	
- due to meningeal involvement	2%	
- due to hemolytic anemia	2%	
Deaths related to complications of therapy:		16%
Deaths unrelated to C.L.L.:		30%
		100%

The variable clinical symptoms and findings as well as the pattern of the survival curves led to many attempts to classify the patients, generally in terms of cell counts or patterns of morphology. The biologic value of "staging" for Hodgkin's and malignant lymphoma led to application of similar techniques to help characterize the significance of clinical findings. The signal application of such staging and corroboration of its clinical applicability was carried out by a consortium study (26) and this pattern of staging has now become standard:

TABLE III

CLINICAL STAGING OF C.L.L.

Stage 0	Lymphocytosis of blood and bone marrow - only
Stage I	" " " " " plus lymphadenopathy
Stage II	" " " " " plus " and splenic and/or hepatic enlargement
Stage III	" " " " plus anemia (Hb <11 g/dl; hematocrit <33 vol%) - [regardless of cause]
Stage IV	" " " " plus thrombocytopenia (platelets <100,000/µl)

The criteria of lymphocytosis in this study were an absolute lymphocyte count of 15,000/µl and marrow lymphocytosis of >40%.

In Stage II nodes may or may not be enlarged; similarly in Stage III and IV lymphadenopathy or hepatosplenomegaly may or may not be present without altering Stage.

Such a staging approach has proved to be a reliable predictor of survival, and its application is beginning to help clarify previous therapeutic adventures and a large literature on "issues in the clinical manifestations of C.L.L."; validity of this type of staging has been amply corroborated in other recent applications (27, 28).

Two potential or anticipated clinical issues in C.L.L. are so rare that they merit special comment:

1.) Leukostasis: In virtually every form of leukemia except C.L.L. a biologic risk to survival exists when white cell counts are elevated. Indeed, physiologic impairment in the CNS and lungs is common with white counts in excess of 100,000/µl in all leukemias except C.L.L. (29). Leukostasis is so rare in C.L.L. that, as noted above, the level of the WBC is of no biologic/clinical significance.

2.) Acute leukemic transformation: Unlike the issue in chronic granulocytic leukemia where acute leukemic transformation is a part of the natural history, the conversion of C.L.L. to an acute form is so rare that it merited report in the literature. In one recent series of 189 patients, 3 conversions to acute leukemia were seen (16). Since overall survival in C.L.L. has not changed during recent decades and since a few (perhaps 1-2%) such conversions are now being seen, a very serious consideration expressed by most investigators is that the acute leukemia occurring in these patients actually represents a second neoplasm, perhaps the result of use of therapeutic agents with carcinogenic potential, as we'll consider later.

The composite of the clinical variation, the data from staging and survival, and the special issues in C.L.L. force the consideration that C.L.L. represents several forms of one disease, a disease entity with a variety of phases or that different subsets of cells (lymphocytes) are the basis for the differences. In the pursuit of biologic definition of C.L.L. a variety of interesting observations (epidemiologic, biochemical, pathophysiologic and immunologic) have slowly broadened our understanding of C.L.L.

V. EPIDEMIOLOGIC OBSERVATIONS:

Although C.L.L. is the most frequent form of leukemia in the U.S. our data concerning etiology/epidemiology/demography are scanty.

A. Demography:

C.L.L. represents 25-40% of all cases of leukemia in most U.S. centers. Since C.L.L. is a disease of "later" life (>40) populations with a short life expectancy can be expected to have a low incidence of C.L.L. Such age patterns have long been used to explain the low incidence in the Far East (in India 5-7% and in Singapore Chinese 2% of the leukemias are C.L.L.). Recent studies (30, 31) have documented that these differences are not merely age related but do relate to geographic differences. Thus, where comparable age groups were evaluated the incidence of C.L.L. is 7.3 times greater in Europeans than in Asians.

B. Familial Leukemia:

In 1929 Dameshek reported twin brothers (age 56) who died of C.L.L. within 68 days of one another; and 25 years later a son of one of the twins (at age 53) developed C.L.L. (32). A comprehensive study of hereditary factors in C.L.L. was reported by Videbaek in Denmark (33) who reported a familial incidence of 8.1% among relatives of 209 patients with C.L.L. (compared to incidence in a control group of 0.5%). Gunz et al. (34) studied 909 families with leukemia and noted: the disease in first-degree relatives was 3 X that of the general population and even in distant relatives it was increased by a factor of 2.3; consanguinity did not appear to be a factor; no known laboratory parameter identified individuals at risk; and, common environmental exposures could not be found. Although such familial susceptibility has been proposed to be due to inheritance of a specific class of lymphocytes (35), the actual data is only speculative as to the genetic issues.

C. Etiologic Studies:

Radiation, an important etiology for other leukemias, is not a factor in C.L.L. Serial evaluation in Japan by the Atomic Bomb Casualty Commission provided further evidence that the incidence of C.L.L. did not change in any population group (36, 37).

Although no known environmental exposure has been identified, it has been suggested by the evidence that although Japanese in the U.S. have a lower incidence of C.L.L. than non-Japanese, the incidence is still greater than that seen in Japanese in Hawaii and both levels are much higher than on the Japanese mainland (30). Other etiologic/epidemiologic studies have not provided data of note in C.L.L. (38, 39, 40).

D. Chromosomal Abnormalities:

Neither clonality nor chromosomal abnormalities have been identified in C.L.L., primarily because of the paucity of mitotic figures for study. In 1962, Gunz described a Christchurch (Ch^1) chromosome abnormality in C.L.L. in a New Zealand family that was seen in cultured skin cells and lymphocytes. It is now clear that this abnormality is a familial constitutional one unrelated to C.L.L. (41).

Current interest in B cell neoplasms have demonstrated 14q+ marker chromosomes in many, and some evidence exists that this is true in C.L.L. as well (42).

VI. BIOCHEMICAL OBSERVATIONS:

In spite of the extensive evidence of heterogeneity of C.L.L., the potential availability of large numbers of cells for study has produced an extensive biochemical literature. Unfortunately, these are descriptive views of cell populations that are almost certainly all heterogenous in any given patient studied and probably reflect different percentage subset populations when different patients are compared. In most circumstances, the changes have not been related to cellular division (let alone neoplastic transformation), to the stage of cellular differentiation or to any other referable stable/standard criteria for appropriate comparison.

Some of the better biochemical parameters are recorded in Table IV. Attention to subset cellular populations is lacking even in the most current studies (52). One observation has been variation in patient to patient (52).

Another approach to cellular biochemical parameters has been to evaluate the effect of a therapeutic intervention in the categorization of potential subset populations or clinical forms of C.L.L. In this laboratory, measurement of cellular ribonuclease activity and the response to steroid therapy suggested one method to segregate patients into clinical subsets (53, 54) and this resulted in a means of identifying steroid responsive C.L.L. patients (53).

Finally, one other form of biochemical observation is beginning to provide a family of leukemic cell markers. A variant alkaline phosphatase (now designated N-alkaline phosphatase) with unique properties (unique catalytic properties and inhibition by cysteine-S-phosphate) has been described in lymphocytes isolated from 13 patients with C.L.L. (55). The basis for this change (i.e. synthesis of new protein, conformational change of the enzyme, altered subunit assembly, allosteric effects, dissociation-association changes, or change in metal ion effect, etc.) is not known. It is clear that the advent of this altered enzyme is not due to high lymphocyte numbers, or altered DNA synthesis (55). It has not been seen in normal lymphocytes or mitogen stimulated "blast-transformed" cells. In addition, it has been reported in both B and T cell forms of C.L.L.

VII. PATHOGENETIC OBSERVATIONS:

The absence of important etiologic data in C.L.L. has led to primary focus on potential "patho-mechanisms" of disease.

TABLE IV

BIOCHEMICAL CHANGES IN C.L.L.

Cellular Site	Enzyme	Lymphocytes From Whole Blood (≈15% B Cells)	Other Normal Lymphocyte Populations				"B" Cell" C.L.L.	"Prolymphocytic" C.L.L.	"T Cell" C.L.L.	Ref.
			Mitogen Stimulated	Tonsils (≈50% B Cells)	Isolated Cells T	Isolated Cells B				
Surface:	Glucocorticoid receptor activity	low (<0.1 pm/mg/prot.)	↑ 5-20x				2 populations of cells: steroid sensitive: hi receptor content steroid insensitive: low content			43
	5'Nucleotidase	0.1-0.9 (μm/hr/mg prot.)			↓		↓ In 10% of cases levels ↑	↓	N-↓	44, 45,46
	γ Glutamyl transpeptidase	N		↑	↓		↓	↓	N-↑	
	Leucine aminopeptidase	N		↑	↓		↓	↓	↑ or ↓	44
	Maltase	N		↑	↓		↓	↓	N or ↑	
	Trehalase	N		↑	↓		↓	↓	N	
Cytoplasmic	Adenosine deaminase	(3200 nm/hr/mg prot.)	↑	3300	2400	1500	700	-	5000	47
	G6PD	N	↑				↓			48
	Polyribosome synthesis	N	↑				↓			49
Nuclear and Nuclear Synthetic Functions	Thymidine kinase	N	↑				↓	-	-	50
	Pyrimidine nucleotide enzymes: - Cdr deaminase	N					↑	-	-	51
	"DNA synthesis: ^{3}H-Tdr uptake	$N_{(Low)}$	↑		N	-	↓	-	-	52
	Poly (adenosine diphosphate ribose) polymerase (DNA replicase assay)	$N_{(Low)}$	↑		N	-	↑ (2.5x that of normal)	-	-	

A. Lymphocyte Kinetics and Traffic:

1. Proliferation kinetics: In most neoplastic lesions the question of the mechanism of mass (cell) expansion is resolved by the increased mitotic activity for that given tissue unit. That is, normal tissues show a progression to cellular "maturation" or "end cell type" that has a very low or absent mitotic rate. The failure of the neoplastic mass to achieve that "mature" cell functional state provides a continued increased mitotic rate for the tissue and usually a less differentiated or mature cell component. In C.L.L. the identifiable compartment is actually the mature cell with a [presumed] low mitotic capacity. Thus, a long standing question in C.L.L. is the mechanism of expansion of the lymphocyte population. Virtually all of the studies of the proliferation kinetics in C.L.L. antedate our knowledge of lymphocyte function, subset populations or even the variant forms of C.L.L. and must be considered with caution. The available data indicates:

- In untreated patients lymphocyte population expansion has been found to be expressible as a straight line in a logarithmic graph describing an exponential increase with a constant doubling time of cells in each phase of disease (56). Even after therapeutic intervention, the increases in cell numbers are lines of almost congruent steepness (57).

- Calculated doubling times are between 4-19 months (16, 57).

TABLE V

LYMPHOCYTE KINETICS-NORMAL VS CLL*

	Normal	C.L.L.
Lymphocytes (µl)	2,200	100,000
Production (µl/day)	46	600
"Short-lived" lymphocytes (µl)	200	5,000
Production - short-lived type (µl/day)	41	540
Average life span - short-lived type (days)	6	5
"Long-lived" lymphocytes (µl)	2,000	95,000
Production - long-lived type (µl/d)	5	60
Average life span - long-lived type (years)	1	5

*(57, 58, 59)

- The normal daily new production rate is 0.2-1% or 300-1500 cells (59).

- In C.L.L. lymphocyte production rates are increased 15-fold (58, 59). Most of this increased production is due to the 25-fold increase in production of "short-lived" lymphocytes. This short-lived lymphocyte population has a normal in vivo survival. A major contribution to the expanded lymphocyte population is due to the increased production (approximately 10-fold increase) of "long-lived" lymphocytes and a 5-fold increase in the average life span (57, 58, 59).

- The isotopic kinetic studies suggest that the production of lymphocytes in bone marrow amounts to approximately one-third that of the lymph nodes (59).

2. Circulation kinetics: Gowans and co-workers' (60) animal studies provide evidence of lymphocyte "re-circulation":

- re-circulation pathway is blood through white pulp of spleen and back to blood; or blood into lymph node cortices and then to blood via efferent lymph circulation; or from blood to tissues and then back by afferent lymph circulation.

- Normally, the extravascular readily accessible pool (RALP) is 25-fold that of the intravascular pool. In C.L.L. there is a shift to the intravascular compartment, so that the RALP is only 3-5 times greater than the intravascular pool (61, 62).

- recirculating cells show normal B and T cell pattern/function (63). The change in pools in C.L.L. has been considered to be due to an altered ability to leave the vascular system and re-circulate (57). The ability to re-circulate has been considered to be a cell surface property (62).

Thus, in general terms C. L. L. has been shown to be associated with increased proliferation of (at least) certain subsets of cells, with cellular accumulation that may be due to such shifts in populations and with alterations in vascular-tissue traffic.

B. The "Morphology" of C.L.L.:

Neoplastic lesions are usually identified by well defined morphologic criteria (organ, cellular, and/or subcellular). The application of "morphologic study" to C.L.L. has resulted in a literature about the "malignant lymphocyte", and most clinical and/or pathologic

reviews tried to use the lymphocyte as the starting point. The problems associated with such an approach neatly is shown by a review of 190 cases from the University of Michigan by Frank Bethell who concluded that the morphological/architectural changes of the lymphocytes documented the true leukemic status of C.L.L. (64); in a back-to-back report, Bruce Wiseman reviewed 66 similar cases from Ohio State and "demonstrated" that C.L.L. was not a neoplastic disease but was a metabolic one (65). The frustrating feature of the pursuit of the "morphologic" lesion of C.L.L. has been the limited structural expression of the lymphocyte, the variety of clinical patterns and the lack of correlation of structure and function (66, 67, 68). The recent knowledge of lymphocyte function has provided the evidence that the mature (appearing) lymphocyte is really part of a functionally heterogenous population.

Although no morphologic description presently defines the biologic expression and variability of "C.L.L.", the newer knowledge of lymphocyte function and traffic has been used to provide a more rational approach to structural characterization. The generalities used to begin this morphologic re-definition are:

- zonal tissue areas are identifiable in lymphoid tissues in which T or B cells are concentrated: B cell zones are the lymphoid follicle (including the germinal center and its peripheral mantle) and the medullary cord areas. T cell zones are the interfollicular or paracortical sites.

- the pattern of B cell response to antigen can reasonably be inferred to consist of a (cleaving-type) alteration in shape and an increase in size: The proposed sequence is the lymphocyte → small cleaved → large cleaved → small non-cleaved → large non-cleaved cell. Cell division is at non-cleaved stage and the cells then migrate to medullary areas where differentiation to mature plasma cells takes place.

- the germinal center provides the cellular amplification needed for the "antigen-activated" B cell.

Albeit slightly circular, C.L.L. has been used as the model to support the "new morphology" by the finding in this disease of mature lymphocytes, monoclonal surface immunoglobulins.

The histology of the nodal tissue in C.L.L. is classically that of a diffuse well-differentiated [lymphoma](1). Unfortunately, the "new morphology" must still acknowledge the following problems:

1. Although diffuse well differentiated lymphoma (WDL) is the tissue manifestation of C.L.L., WDL may exist as a distinct form of non-Hodgkins lymphoma or as a monoclonal gammopathy usually of the IgM type (i.e. macroglobulinemia), in the absence of C.L.L. (1).

2. In some patients the tissue morphology has been classified not as WDL but as intermediate cell type (70). Again, not all intermediate (lymphocytic lymphoma) cell forms are associated with C.L.L. and when classified only on cellular architecture there does not appear to be a biologic difference in this pattern. When the tissue mitotic activity is used as the critical "morphologic" criteria of this intermediate cell type and when it exceeds 30 mitotic figures per 20 high power fields, this morphologic variant has a poor prognosis (70). This latter statement applies to those with or without C.L.L.!

3. When the morphologic patterns and variants are classified by standard light microscopy methods, little agreement can be found regarding the relationship of morphology to clinical course or survival (13, 71, 72, 73). It has been suggested that the ratio of the percentage bone marrow space occupied by lymphatic tissue (in section) to the absolute peripheral blood lymphocyte count relates to survival (72); others have not corroborated that type of tissue pattern (73).

4. In addition, two separate facets of "tissue inconstancy" must be clarified:

a.) The advent of a superimposed or second hematopoietic neoplasm, such as acute leukemia (74, 75, 76, 77, 78) or Hodgkins (79) has posed confusion. As mentioned above, all of the current evidence is compatible with the advent of the second lesion by the same carcinogenic influences which affect the general population; that is, these appear to be separate malignant processes unrelated to the C.L.L.

b.) Richter's syndrome: In 1928, Richter described a 46-year old man with a classical picture of C.L.L. (SBC of 98,400/µl). With little explanation, the patient suddenly had a rapidly progressive course and at autopsy two different lesions were noted: those with WDL and a second more primitive involvement of lymphoid tissue labeled reticulum cell sarcoma (80). This "tissue inconstancy" has been extensively reviewed (66, 81, 82, 83) and many of these cases have in the past been incorrectly called Hodgkins disease or histiocytic lymphoma.

TABLE VI

FEATURES OF RICHTER'S SYNDROME

1. Initial presentation of C.L.L.

2. Rapid onset (without clinical cause) of constitutional symptoms, weight loss, localized lymphadenopathy, lymphocytopenia and abnormal immunoglobulins in serum or urine.

3. Histologic evidence of both WDL (or a C.L.L. compatible lesion) and a pleomorphic malignant lymphoma with prominent multinucleate tumor cells.

The demographic data, clinical course and therapy responsiveness have clearly shown that Richter's is not a transitional form to Hodgkins or other heretofore defined lymphoma (81, 82, 83). The cellular inconstancy in lymphoid neoplasms has been extensively reviewed. In autopsy series of malignant lesions of the lymphoid system, 30% of cases may show such inconstancy; even in a current clinical series Kim and Dorfman had a 16% incidence of such inconstancy that it led to the term "composite" lymphoma. The mechanisms for such composite lesions are speculative. In C.L.L. some authors (82) have compared the development of Richter's to the "blast transformation" of chronic granulocytic leukemia.

5. Finally, the concept of "spill-over" leukemia in the malignant lymphomas must be clarified. In 1937, Isaacs utilized the term "lymphosarcoma cell leukemia" to describe the circumstance in the "natural history of a malignant lymphoma" when a leukemic phase was seen (84). As he pointed out, Sternberg had suggested this in 1908 to help define the hematologic extension of poorly differentiated lymphoma. Such a (late or terminal) leukemia transformation has been seen in 10-20% of all carefully studied large series of patients with malignant lymphoma (85, 86, 87, 88). Although Isaacs suggested nucleolar changes on supravital staining as the "morphologic marker" (84), the greatest success at identification has been where the cleaved cell pattern (of tissue sections) is seen in the peripheral blood. In essence, this diagnosis is best made in the setting of existent lymphoma. The appelation "spill-over" leukemia is a recent re-emphasis of this pattern, in order to dissociate these events from the attempts of Linman and co-workers to categorize a large subset of C.L.L. patients as chronic lymphosarcoma leukemia (69, 89). Little clinical, laboratory or biologic data exists for the Linman proposal and it should be dropped.

C. Functional Alterations in Lymphocytes:

The rapidly exploding knowledge of lymphocyte function has provided a parameter of comparative reference for the cells found in patients with C.L.L. and has provided an important approach to recognizing and characterizing the complex cellular populations involved and providing techniques for exploration of cellular differentiation in the lymphoid system:

1. Lessons from immunobiology relevant to C.L.L.:

a.) Evidence of decreased mitogen responsiveness (diminished rate and delayed time course) of cellular transformation (90, 91); those blasts produced are no different than those produced from cells isolated from normal individuals (92). That this altered responsiveness is not just due to different cellular populations (see below) but may represent a truly altered membrane is suggested by defective responsiveness to pokeweed stimulation, a B cell stimulant (93).

b.) The identification of functional classes of lymphocytes (94) was promptly followed by the evidence that C.L.L. was (primarily) a B cell disease since immunoglobulin could be identified on the surface of the lymphocytes (95). Although the percentage of immunoglobulin-positive cells did not correlate with the degree of lymphocytosis or stage of the disease, the characterization of the surface immunoglobulins (SIg) and other surface receptors has provided evidence of clonality in C.L.L. and these moieties have become the probes for studying differentiation (94, 96, 97) and have yielded:

1.) The majority of lymphocytes in patients with C.L.L. have surface binding sites for markers of B cells (cell surface immunoglobulins, aggregated IgG, and immunocomplement complexes).

2.) Differences from normal lymphocytes exist; for instance, the phenomenon of "cap formation" (a polar redistribution of receptors on the outer membrane) is absent in C.L.L. (98).

3.) Most cases of C.L.L. have IgM and/or IgD on the surface of the lymphocytes, with a single light chain type (i.e. κ or λ). Other patterns have been seen: Ig retained as intracytoplasmic crystals (99), or light chain inclusions (100).

4.) In patients with C.L.L., a small percentage of normal B cells are present (101) helping explain why agammaglobulinemia rarely occurs. Similarly, a small normal T cell population also appears to exist in C.L.L. (102). These normal populations may be critical in modulating the severity of the clinical expression in C.L.L.

5.) Preliminary observations of SIg suggest some clinical prognostic relevance to the identified surface membrane marker:

TABLE VII

SURFACE Ig AND CLINICAL FINDINGS

SIg-G:	occurs rarely; commonly associated with more aggressive disease.
SIg-M and SIg-D:	most common; "classical" C.L.L.
SIgM:	rare; associated with more benign clinical course (103).

2. Identification of lymphocyte population subsets:

It is clear that a variety of lymphocyte populations exist. Important class subpopulations are now known as well as a heterogenous third population ("null") of lymphocytes (104, 105). That such subpopulations are identifiable in C.L.L. is now clear (106, 107, 108, 109). In some circumstances selected therapeutic intervention, such as glucocorticoid effects on T cell subpopulations (110), has been exploited in the characterization of cell subsets. The preliminary data to date has not afforded enough information to comment on the clinical importance of such subpopulation studies (111).

3. Lymphocyte differentiation:

The studies of Vitetta and Uhr (112) provided important recognition of the existence of lymphoid differentiation markers. Indeed, the sequence of cellular differentiation and maturation has been extensively defined in the mouse (112). Although the corollaries are expected in man (113), to date the data is too scanty to provide anymore than mere speculation. Admittedly, one way to explain some of the variation in the forms of C.L.L. would be in relationship to degrees of differentiation. Studies of the effectors of lymphoid differentiation (114) suggest that the lymphoid mass is a marvelous experimental model because many factors affect differentiation "in vitro"; unfortunately, the "in vivo" correlates are scanty.

4. Cellular population analysis:

A major advance of the past few years has been the development of techniques making it possible to isolate individual cells from heterogeneous populations. The most significant advance in this regard is the development of the Fluorescent Activated Cell Sorter (FACS) which provides a unique opportunity to analyze heterogeneous populations of cells according to their optical properties and then mechanically separate individual cells from that total population for further selected study (115). The FACS technology has provided remarkable speed having the capability of processing 100,000 cells per second, as well as phenomenal sensitivity due to its laser optics which provide a pure spectral stimulation. The system can be used with a variety of fluorescent antisera that allow the recognition of selected elements on the cell surface. The ability, then, to sort, separate and isolate individual cells from a population of cells permits detailed investigation of a selected component of an otherwise heterogeneous cellular population. The individual isolated cells can then be studied further for various aspects of their functional capacity.

The ability to analyze, sort and separate cellular populations by these techniques of light scatter and fluorescence permits a wide variety of applications. For instance, in addition to the analysis and separation

of lymphocyte subpopulations according to their immunologic function that we will presently describe, the same techniques can be used for cell-cycle analysis by the measurement of fluorescently labeled DNA, RNA or protein; characterization and measurement of a variety of intracellular enzymes; analysis and sorting of individual chromosomes; studies of circumstances of selected lectin binding; characterization and enumeration of live cell/dead cell populations; or, specialized studies of the cellular immune response.

Since the cell sorters are equipped with the potential for multi-unit analysis, the FACS can identify individual markers of different types and analyze them at the same time. In essence, this technology provides a new approach to a multi-dimensional picture of a given cellular population. The result of such a study of multiple cellular components is a rapid definition of the "fingerprint" of that given cell or subset population.

VIII. CLINICAL FORMS OF C.L.L.:

The recognition of different lymphocyte populations has aided the use of clinical, hematologic and immunologic definition of the clinical forms of C.L.L. Four major leukemic forms can be defined as variants of C.L.L. with the current data:

- B cell C.L.L. (classical C.L.L.)
- T cell C.L.L.
- Prolymphocytic leukemia
- Lymphosarcoma (or "spill-over") leukemia

Some of the important clinical features are shown in Table VIII. Although initially the T cell variant was considered actually to be expressed as a cutaneous form of disease, it is now quite clear from the brilliant studies of Brouet and co-workers (116) that a rather typical clinical picture of T cell C.L.L. exists (117, 118, 119, 120). The lymphocytes that characterize the T cell lesions are large, very uniform with considerable cytoplasm and contain large numbers of prominent azurophilic granules. Although a clinical relationship to Sézary's has been considered, the cytology and ultrastructure are different (116, 120). A high content of β-glucuronidase and acid phosphatase is seen in greater than 90% of the peripheral blood lymphocytes (116). Blood and bone marrow involvement are generally modest; for instance, most patients have had only 30-70% lymphoid cells in bone marrow specimens.

TABLE VIII

FEATURES OF THE CHRONIC LYMPHOCYTIC LEUKEMIC FORMS

	B Cell C.L.L.	T Cell C.L.L.	Prolymphocytic Leukemia	Lymphosarcoma (Spill-over) Leukemia
Age at Dx	Rare <40	All ages	Rare <60	All
Male:Female	2:1	1:1	7:1	1:1
Constitutional symptoms (fever, wt. loss)	Uncommon (20%)	Uncommon	All (100%)	Common (>90%)
↑Lymph nodes	Common; large	Rare & small	Rare	Common
Mediastinal lymphadenopathy	Common	Rare	Not seen	Common
Splenomegaly	Frequent	Usually massive	Usually very massive	Common; modest
Skin lesions	Rare	Common	Rare	Rare
Anemia (<11g)	Late in course	Common	All	Common
WBC per µl	Variable	Usually 3-50,000	Usually >200,000	Usually 10-50,000
Lymphocytes	Mature; some heterogeneity	Very uniform; large cytoplasm & azurophilic granules	Large, fine chromatin, large nucleolus	Frequent "cleaved-forms"
Immunologic markers	B cell, esp. IgM-IgD	T cell	Both B and T cell forms; B cells often intense SIg	B cell; -Intense SIg -cap formation
Serum/urine proteins	Hypogammaglob. ≃25% M spike ≃10-15%	Polyclonal-gammopathy common	Normal	-
Response to cytoreductive therapy	Very good	Good	Poor	Good; often transient
Survival (median)	3 1/2 years	Variable (like B cell)	4-5 months	Variable

Another variant group was termed "prolymphocytic" C.L.L. by Galton, Catovsky and their co-workers (121, 122, 123, 124, 125). Clinically these patients appear with constitutional symptoms that suggest a "subacute" leukemia. They are elderly males with massive splenomegaly and very high white counts, cytocellular histologic features that help in diagnosis. Their median survival is short (17 weeks) and only 5 of the initial 15 survived one year (121). Recent therapeutic trials have reaffirmed a relatively modest response to the usual therapeutic approaches for leukemia, including splenic radiation. By contrast, good responses have been reported with leukapheresis and with splenectomy (124, 125).

Finally, the recent studies of surface markers have helped reaffirm the classical concept of lymphosarcoma cell leukemia previously discussed as a leukemic phase in a patient with a previously diagnosed malignant lymphoma (126, 127, 128). The laboratory features of very intense fluorescence when the lymphocytes are stained with the fluorescein-labeled anti-immunoglobulin and cap formation during incubation help separate these cases from classical B cell C.L.L. (128).

The definition and characterization of these clinical forms provides an updated and helpful "working approach" to the patient problems. The same questions continue to exist: - Are these leukemic lesions simply the expressions of an arrested sequence of differentiation (e.g. from lymphoid precursor → lymphocyte → plasma cell, for instance); are the lesions simply defects in differentiation (129, 130)?

- If the lesions are arrests of the differentiation sequence, might one expect C.L.L. to be a more common terminal event in patients with malignant plasma cell dyscrasias? Its rarity merited the recent first such case(131).

- Are all of these neoplastic lesions (including the related lymphomas) simply just phases of a single disease (6)?

- Does the "composite" lesion, Richter's syndrome, the occasional acute leukemic event and similar changes indicate that the "C.L.L." event is only a tissue response to the primary evolving "truly malignant lesion"?

These questions and a variety of extensions have not been answered by the recent findings described above. Indeed, no one model of disease adequately answers the questions posed by the clinical and laboratory events in C.L.L. and its variant expressions.

IX. THERAPEUTIC APPROACHES AND STRATEGIES:

Comparison of C.L.L. survival data from the 1920's (13) to the current era (26, 132) reveals a "5-year survival fraction of 55%" and suggests that our contemporary modes of therapeutic intervention have not affected the natural history of C.L.L. The relative benignity of C.L.L. (as leukemias go) and the occasional "spontaneous" cure (133) notwithstanding, when one evaluates patient survival by stage of disease (26) or in some of the clinical forms (i.e. prolymphocytic leukemia) it is clear that altered therapeutic strategies are needed. Traditionally the indolent cases of C.L.L. have not been treated; those with symptoms or advancing cell burdens have been treated with the cytoreduction measures available or in vogue during that era, as shown in Table VIII.

In most of the therapeutic trials the clinical stage of the patient is not defined. In virtually none of the trials is the form of C.L.L. characterized. In most studies the intervals used to calculate survival are not clear (i.e. from onset of symptoms, first clinical visit, initial diagnosis, institution of therapy, etc.) and the populations are very heterogeneous (134). Finally, no C.L.L. uses appropriate clinical trial design and analysis methodology (135, 136).

The therapeutic trials to date can be categorized as follows:

A. Radiation:

Historically, radiation therapy was the initial mode of treatment for C.L.L., and as the technology of radiation delivery advanced so too did the therapeutic trials:

1. Total Body Irradiation (TBI):

a.) Classical trials are represented by the del Regato series of 61 patients extending from 1943-1969 (137). Treatment series: 10 daily irradiations of 10r; one weekly irradiation of 5r; regional irradiation of spleen or lymph nodes as required; an annual "booster" of 10 daily irradiations of 10r.

b.) Current TBI has been "re-pioneered" by Johnson and co-workers during his tenure at NCI (138). Fractionated therapy: 5-10 rads per day (3-5 X per week) to 200-400 total dose. This series is unique in that 33% of patients achieved a complete remission and in this group 65% of patients were alive at 6 years.

2. Total Body Radiation with Radiophosphorus:

a.) John Lawrence introduced artificially produced radioactive phosphorus (^{32}P) for the treatment of chronic leukemia in 1936 and his first clinical results were reported in 1940 (139). His series (1936-1960) included 161 patients treated with 4 to 6 injections at weekly intervals to a total dose of 3 to 8 millicuries (140).

b.) Edwin Osgood fractionated ^{32}P therapy and treated 212 cases of C.L.L. using approximately 2 millicuries (depending on initial WBC) for induction and then 0.3 to 1.0 mc for maintenance at 4 to 12-week intervals (141). A good median survival was achieved, but induction of a complete remission was not a therapeutic criterion.

c.) Joe Hill at Wadley used ^{32}P in a colloidal suspension (colloidal zirconyl phosphate) in the treatment of C.L.L.; a very long median survival was achieved (90 months), but only 13 of the 97 patients were treated with ^{32}P alone (142).

3. Local Radiation:

a.) Splenic irradiation has generally been used for "symptomatic" splenomegaly. A recent evaluation (143) of its systemic effect was carried out in 14 patients treated with splenic midplane doses of 200-1750 rads (over 3-14 days), usually 300-450 rads given in 2 or 3 fractions of 150 over 3 to 8 days. The patients had a long average interval from the onset of C.L.L. to their radiation (38 months). As expected, they had an excellent local response (decrease in pain and spleen size). In addition, in 11 of 23 courses of radiation in this group a 75% or greater reduction in circulating white count was achieved. The duration of clinical responses was quite variable but an average of approximately 18 months' stability was achieved (143).

b.) Thymic irradiation has been under study by a Winston-Salem group since 1963 (144) using 3000 rads to mid-plane mediastinum through anterior and posterior ports over a 4-week period, 4 times per week. They have classified their responses according to clinical stage of disease and obtained an overall "full remission rate" (a term used in place of CR because marrow repair was not recorded) of 40% (145). The number of patients in each stage is too small for a meaningful determination of effect on survival. That this was a symptomatic group is suggested by the need for further therapy in most patients within 2 years; nevertheless, 8 (of 40) patients required no other therapy for periods of 28 months to 13 years (145). Other trials have not been so positive. Sawitsky treated 31 patients (146) and achieved only transient responses in about one-half the cases.

B. Leukapheresis:

Cytoreduction by cell removal has not been extensively applied (147). Since lymphocytes are more buoyant than PMN's, leukapheresis is a very efficient way to remove lymphocytes, with collection efficiency of 60% (compared to 30% for PMN's). In most patients studied, cellular removal was associated with decrease in mass disease and clinical stability (147). Long term studies are not yet available. As mentioned above, the European experience suggests that cell separation is the preferable approach in prolymphocytic leukemia.

C. Adrenal Corticosteroids:

The application of adrenal corticosteroids to lymphoid neoplasms in man occurred after the observed decrease in lymphoid mass in animals (148). Careful clinical trials were not performed, but the early evidence (130) of an improved sense of well-being and a decrease in the severity of the warm antibody hemolytic anemia were commonly observed (149). In a long term study at Roswell Park (150) the inability to induce complete remissions and the transient nature of the clinical responses were confirmed. In general, steroid therapy (usually in daily doses of 20-60 mg prednisone-equivalents per day) results in:

- Increase in sense of well being; improved appetite; loss of constitutional symptoms.
- Generally (approximately 80%) a marked decrease in lymphoid mass, especially spleen but also lymph nodes.
- Increase in hemoglobin/hematocrit values.
- Increase in platelet counts.
- Decrease in existent warm antibody hemolytic anemia.

An unexplained finding in about three-quarters of patients is an abrupt rise in circulating lymphocytes. This discordant effect (decrease in mass disease and an increase in circulating lymphocytes) has been considered to be a redistribution effect. Abundant evidence (130) proved this to be incorrect, although the mechanism for the increased "leukemic" response in the blood is not known. All of the studies demonstrate that as a "single agent" form of lympholytic therapy the responses are not durable in that 70% of cases had evidence of recrudescence of findings within 4 weeks of stopping therapy (149, 150).

Finally, it should be noted that high dose, one-time-per week steroid therapy has been successfully used in patients who have become refractory to all other agents and/or modes of therapy (151).

D. Cytotoxic Agents:

1.) Alkylating Agents: Every alkylating agent developed has been used to treat patients with C.L.L. and "response rates" for single agents have been between 40-80% (134, 152). This extensive literature has been reviewed (134, 152) and in general:

- response rates of 40-80% are in part due to different criteria of "response".

- chlorambucil, first introduced in 1952, is the most commonly used oral alkylating agent with cyclophosphamide a close second in the U.S. Chlorambucil response rate (60%) may be higher than that of cyclophosphamide (45%).

- complete remission rates are at best 10%. This may be increased to nearly 20% when combined with adrenal steroids (153).

- other alkylating agents are capable of cytoreduction, but appear to be less effective and have a higher degree of toxicity (e.g. long lasting platelet toxicity with busulfan [Myleran]).

- no evidence exists that the two plus decades of alkylating agent therapy has affected the survival data in C.L.L.; most agree that the "quality of life" is better (154).

2.) Combination Chemotherapy:

Most of the combination trials to date have employed an alkylating agent (usually chlorambucil or cyclophosphamide) plus prednisone which appears to increase slightly the CR rate.

a. CVP (Cytoxan-Vincristine-Prednisone): In one series of 36 patients carefully staged a 44% CR was achieved with:

Cytoxan: 400 mg/M^2/d (O) day 1-5
Vincristine: 2 mg I.V. day 5
Prednisone: 100 mg/M^2 (O) day 1-5

- re-treatment q. 3 weeks until maximum response (usually 8 courses); following this induction, CR were re-treated q. 6 weeks for maintenance; partial responders were continued on above to toxicity or CR. Although this is a small series, patients were carefully staged pre and post therapy and remission criteria were unambiguous. Median survival has not been reached but actuarial survival curves reveal:

1. a clear survival difference between responders and non-responders.
2. actuarial projection of prolonged survival in the complete responders.
3. Finally, these studies are particularly important because they provide clear evidence that the induction of a complete remission in C.L.L. is possible and that such an event has survival advantage.

TABLE IX

THERAPEUTIC RESULTS IN C.L.L.

Mode of Therapy	Number of Cases	% Achieving Complete Remission	% Alive 5yr.	% Alive 10yr.	Median Survival (mos.)	Dates of Study and Comments	Reference & Author
I. Radiation: Total body	61	-	21	10	39	1949-1969	(137) del Regato
	80	33	58	-	57	1967-1976	(138) NCI
^{32}P	161	-	60	20	52	1936-1960	(140) Lawrence
	212	-	52	25	64	1941-1954	(141) Osgood
	97	-	-	-	90	1956-1962 (All but 13 Rx with other agents.)	(142) Hill
Local - thymic	40	45	-	-	?	1963-1978	(145) Richards
II. Adrenal steroids	80	0	38	-	26	1950-1965	(150) Roswell-Park)
III. Alkylating agents	Hundreds	10			?	1960-1975	(134;152)
IV. Combination chemotherapy: Chlorambucil plus Prednisone	96	22			?	1970's	(153)
CVP	36	44			>36 mos.	1971-1975	(155)

b. Other combinations appear to be similarly effective but have been used in more limited trials:

1. Multiple Alkylating Agent Program:

BCNU:	0.5 mg/kg I.V.	day 1
Cytoxan:	10 mg/kg I.V.	day 1
Melphalan:	0.25 mg/kg/d, (O),	day 1,2,3
Prednisone:	1 mg/kg/d, (O),	day 1-7.

2. Cyclophosphamine-Cytosine Arabinoside:

Each drug 37.5 mg/M^2 I.V. q. 12 hours
for 4 days

Then courses repeated q. 21 days.

E. Selected Therapeutic Problems and Issues:

1.) Hypogammaglobulinemia (156)

2.) Anemia/Thrombocytopenia: The differential diagnosis of either or both of these can be approached in rational sequence:

a. Blood loss
b. Immunologically mediated increased destruction:
   - autoimmune hemolytic anemia
   - immunologic thrombocytopenic purpura
c. Ineffective hematopoiesis - primarily secondary to nutritional deprivation (especially folate)
d. Enlarged splenic pool - hypersplenism (157)
e. Marrow replacement by the expanding proliferative leukemic cell population
f. Marrow injury secondary to therapeutic intervention

A resurgence of interest in splenectomy (157, 158, 159) has again demonstrated that the best results occur in circumstances of a clearly expanded pool especially when selective splenic sequestration (spleen to liver ratios of 2.4:1 or greater) can be demonstrated by some technique of trapping of radiolabeled cells.

3.) Specific Endogenous Mitotic Inhibitors (Chalone) of Lymphocytes and/or Antithymocyte Antiglobulin:

- no significant therapeutic promise (160, 161, 162)

X. FUTURE THERAPEUTIC APPROACHES:

B cell neoplasms provide an interesting model for therapeutic intervention, since they are monoclonal and bear surface immunoglobulin (SIg) that is not secreted as a paraprotein. In expressing the SIg all of the cells of the tumor have the same constant and variable regions of both the heavy and light chains. The variable region of the SIg provides the tumor cells a degree of uniqueness, since this marker is present on only a limited number of normal cells but on all the cells of the expanded (clone) tumor population. Indeed, the variable region provides the marker or idiotype that is its own "fingerprint". The ability to produce a highly specific anti-idiotypic antisera by the use of the hybridoma provides a heretofore unavailable specific "immunodestructive therapeutic modality". That such approaches are possible in selected experimental systems (163) is now clear. In addition, such an immunotherapeutic approach has "cured" a murine B cell leukemia model that closely simulates C.L.L. in man (164).

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