Studies in chronic lymphocytic leukaemia II. Lymphocyte markers, cellular and humoral immunity and the effect of treatment

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Summary

Observations were made on 15 patients with chronic lymphocytic leukaemia, 3 with non-Hodgkin's lymphoma, and 18 healthy controls. These include characterization of lymphocytes, assessment of humoral and cell-mediated immunity and the effect of treatment. Those responding to therapy showed a disappearance of 'null' lymphocytes from the blood with improvement in clinical and haematological parameters. Their immune capacity, however, remained unchanged or continued to deteriorate.

Introduction

Patients with chronic lymphocytic leukaemia (CLL) have an abnormally high proportion of B-lymphocytes in the peripheral blood. This was shown in CLL by demonstrating immunoglobulin on the lymphocyte surface (Pernis *et al.*, 1971; Wilson and Nossal, 1971; Unanue *et al.*, 1971; Papamichail, Brown and Holborow, 1971; Rabillino and Grey, 1972; Van Boxel *et al.*, 1972a; Preud'Homme and Seligmann, 1972; Ross *et al.*, 1973; Seligmann, Preud'Homme and Brouhet, 1973; Haegert, Hallberg and Coombs, 1974; Seligmann, 1974; Fu and Kunkel, 1974; Kubo, Grey and Pirosky, 1974; Seligmann, 1975), by demonstrating complement receptors on the cell surface (Pincus, Bianco and Nussenzweig, 1972; Shevach, Herberman and Frank, 1972; Jondal, Holm and Wigzell, 1972; Ross *et al.*, 1973; Huber, Michlmayr and Huber, 1974; Cohnen *et al.*, 1974): or by demonstrating lymphocyte binding of aggregated IgG (Dickler *et al.*, 1973; Augner, Cohnen and Brittinger, 1974; Huber *et al.*, 1974).

T-lymphocytes in CLL are diluted by virtue of the

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increase in B-cells (Melief, Schweitzer and Eijssvooge, 1973; Wybran, Chantler and Fudenberg, 1973; Fernandez, MacSween and Langley, 1975) but the absolute numbers are maintained in most cases.

In addition, there are lymphocytes which are neither T-cells nor show the usual reactions of B-cells. These have been termed null-cells. They are also increased in CLL (Nath *et al.*, 1975).

Hypogammaglobulinaemia is present in between 35 and 75% of patients with chronic lymphocytic leukaemia (Jim, 1957; Boggs and Fahey, 1960; Fairley and Scott, 1961; Miller, 1962; Schumann, 1963; Mobarak and Brody, 1967; Buckley and Dorey, 1971; Scamps, Streeter and O'Neill, 1971; Hansen, 1973; Slungaard and Smith, 1974). Many of these patients show an increased liability to infection with both bacteria and viruses (Hansen, 1973). Between 5 and 13% of patients get herpes zoster (Videbaek, 1949; Hansen, 1973). Bacterial infections most frequently involve the respiratory tract.

The present authors report observations on the lymphocytes in patients with CLL and lymphoma, including comparison of cells in the blood and lymph gland, on the results of tests for immunity and the effect of therapy on these observations.

Patients studied

Observations were made on 15 patients with CLL, 3 with lymphoma and 18 normal subjects. The patients with CLL (cases 1-15) varied from those who were well clinically with only an elevated total lymphocyte count, to those with anaemia, marrow infiltration, glandular enlargement and splenomegaly (Table 1). One patient had an autoimmune haemolytic anaemia (case 14). The initial blood count is shown in Table 2.

TABLE 1. Clinical findings in the patients

Patient	Age	Sex	State of health	Lymph-adenopathy	Splenomegaly (in cm below costal margin)	Hepatomegaly (in cm below costal margin)	Marrow infiltration (% lymphocytes)
1	44	M	Good	-	-	-	48
2	65	F	Good	-	-	-	34
3	59	M	Good	-	-	-	47
4	64	M	Good	+1	-	-	35
5	75	M	Good	-	-	-	52
6	55	F	Good	-	-	-	49
7	41	M	Good	+3	7	1	80
8	60	M	Good	+2	-	-	74
9	73	F	Moderate	+3	8	-	98
10	48	F	Poor	+3	3	4	92
11	74	M	Good	+3	10	2	97
12	67	F	Poor	-	10	-	72
13	65	F	Poor	+1	4	-	78
14	73	M	Poor	+3	8	5	65
15	79	M	Poor	-	15	4	78
16	63	M	Poor	-	20	3	78
17	70	M	Good	+3	-	-	-
18	68	M	Poor	+3	12	1	43

- No enlargement, + one enlargement in one zone, +2 enlargement of several groups of glands, +3 generalized lymphadenopathy. Cases 1-15 had CLL; cases 16-18 had lymphoma.

Cases 16, 17 and 18 had a lymphoma of lymphocytic type; in 2 of these, abnormal lymphoid cells were present in the blood. Both these patients died within 4 and 10 weeks of diagnosis. The third patient with lymphosarcoma showed a good response to cytotoxic therapy (mustine, vincristine, prednisone and procarbazine).

Cases 13 and 15 had repeated chest infections and required gammaglobulin supplements; cases 9, 10 and 12 had herpes zoster infection complicated by encephalitis in case 12 and hepatitis in case 10. Chronic emphysema and bronchitis occurred in cases 14 and 15. Gram-negative septicaemia occurred as a complication in case 10 and case 16 with lymphoma.

Five patients died (cases 10, 14, 15, 16 and 18). Follow-up ranged from 1 to 2.5 years. Treatment was with chlorambucil with or without prednisone. Cases with heavy marrow infiltration were treated successfully with a mustine, procarbazine, vincristine, prednisone (MOPP) regime. Five patients remained untreated (cases 1, 2, 3, 4, 6) and one (case 5) required treatment 2 years after diagnosis. Tests were carried out before treatment was given except in case 15 who had had intermittent therapy elsewhere over a period of 10 years. He was not on treatment when tested.

Methods

The methods used were described in detail by Bazerbashi (1975).

Collection of lymphocytes. Between 10-50 ml of blood were taken into heparin or defibrinated.

Two volumes of blood were added to one volume of plasmagel and left at room temperature for 20 min. The supernatant containing lymphocytes was transferred to an equal volume of Ficoll-Triosil mixture and centrifuged at about 600 g for 25 min. Lymphocytes and monocytes were harvested from the interface into 25 ml RPM1 solution and washed twice. In CLL, less than 1% of the cells were monocytes. With normal blood a trace of metallic iron powder was added and after 30 min the monocytes attracted to the bottom of the universal container with a magnet. The supernatant was harvested.

Thinly sliced lymph glands were emulsified against the mesh of a stainless steel wire tea strainer. The suspension was filtered through surgical gauze and washed in RPM1 solution. All suspensions were tested for viability by the trypan blue viability test.

Ficoll-Triosil mixture. Ten parts of Triosil (20 ml Triosil 440 and 24.2 ml water) were mixed with 24 parts of sterile 9% Ficoll solution.

RPM1 pH 7.2. The solution was purchased from Gibco-Biocult Ltd.

Demonstration of surface immunoglobulins. Lymphocytes were suspended in a concentration of 2 to 3 million cells/ μ l, one ml added to each of 7 plastic 1.7 $\times$ 5-cm tubes, centrifuged and supernatant discarded. About 50 μ l of polyvalent fluorescein-conjugated rabbit antiserum against heavy and light chains (Mercia diagnostic laboratories) were added to one tube and unconjugated rabbit antisera against γ , α and μ heavy chains and against κ and λ light chains added to the remaining tubes. The last tube, serving as a control had 50 μ l of swine anti-rabbit fluorescein-labelled serum added.

TABLE 2. Details of blood counts on initial presentation on the patients studied

Case no.	RBC × 10 ⁶ /µl 4.5-7*	Haemoglobin g/100 ml 12.5-17.0	PCV 36-50%	MCV 80-92 fl	MCH 27-32 pg	MCHC 32-36%	Total WBC 4-11 × 10 ³ /µl	Polymorph neutrophils 2.5-7.5 × 10 ³ /µl	Lymphocytes 1.5-3.5 × 10 ³ /µl	Monocytes 200-800 × 10 ³ /µl	Eosinophils 40-400 × 10 ³ /µl	Reticulocytes <2.0%	Platelets 100-400 × 10 ³ /µl
1	8.5	8.91	49.0	85	29.0	34.0	16.1	4.5	14.2	390	390	0-9	205
2	4.20	12.7	39.8	95	30.0	34.5	10.4	3.5	6.7	100	100	2.0	295
3	4.67	15.4	44.2	89	30.9	34.8	19.5	2.6	16.5	162	157	0.8	210
4	10.9	17.4	49.4	82	28.9	35.2	27.1	8.0	18.5	578	-	2.0	165
5	10.9	14.9	41.0	89	32.4	36.3	46.1	4.2	41.8	55	-	1.2	164
9	8.4	15.2	43.0	89	31.5	35.6	19.3	4.0	12.0	163	-	0.6	192
7	8.8	14.7	40.1	83	30.4	36.6	27.3	2.2	24.8	284	-	1.6	126
8	5.52	15.1	47.4	86	30.4	33.3	36.0	5.8	29.0	1032	-	1.6	210
6	6.63	11.1	55.0	89	28.4	7.13	221.0	4.8	215.0	710	400	9.1	545
10	6.93	5.3	14.5	-	14.7	9.93	67.0	2.7	63.5	-	67	4.0	26
11	7.23	12.7	37.2	79	30.0	34.0	58.1	6.5	51.0	578	-	1.7	106
12	4.4	12.3	25.8	55	16.9	30.4	50.2	3.0	47.0	120	60	0.2	288
13	4.14	12.3	35.7	89	26.9	34.0	32.9	5.1	26.9	444	222	1.2	278
14	4.18	9.9	18.2	98	35.3	35.9	18.1	2.0	5.8	243	-	0.8	72
15	4.42	10.7	33.6	76	24.2	30.9	30.8	6.9	24.0	416	-	1.4	178
16	4.20	9.4	28.0	88	29.4	33.6	16.5	2.5	13.8	125	125	3.3	86
17	4.82	14.4	42.1	87	29.8	44.2	18.1	5.5	1.3	405	891	2.0	225
81	6.3	1.6	27.9	75	24.6	9.23	11.1	2.3	8.1	505	180	0.8	68

*Normal range.

After incubation for 60 min at 4°C, the cells were washed 3 times and anti-rabbit fluorescein-conjugated serum was added to the tubes containing monospecific antisera. These were again left for 60 min at 4°C and washed 3 times in phosphate-buffered saline. Finally the cells were resuspended in buffered glycerin (glycerin 1 part, phosphate-buffered saline 2 parts) and examined by ultra-violet microscopy. The total number of cells and those exhibiting fluorescence were noted.

When the great majority of cells showed fluorescence the cells were re-incubated with 0.25% trypsin in RPMI at pH 7.2 to remove adsorbed serum, immunoglobulin or immune complexes, and re-examined.

Demonstration of surface complement receptors. Sheep red blood cells (SRBC) were washed in phosphate-buffered saline and suspended to give a 5% suspension. One in 1000 (sub-haemolytic dose) of SRBC antiserum was added, left at 37°C for 30 min, washed and 1/5 dilution of mouse complement (pooled serum from 10 mice bled by heart puncture) added. The suspension was incubated for a further 30 min at 30°C, washed in phosphate-buffered saline and resuspended in RPMI with 10% heat inactivated and sheep cell-absorbed fetal calf serum to give a 5% suspension.

Two million lymphocytes in 0.5 ml were mixed with 0.5 ml sensitized sheep cells and rotated for 20 min at 37°C. A drop was transferred to a white cell counting chamber and examined. Lymphocytes surrounded by not <4 sheep cells were regarded as forming rosettes. The control preparation consisted of sheep cells without complement.

Spontaneous sheep-cell rosettes. Lymphocytes were suspended to give a concentration of 5×10^6 /ml. Sheep cells were suspended to give a 2% suspension; 0.1 ml lymphocytes, 0.1 ml inactivated and sheep cell-absorbed fetal calf serum and 0.2 ml sheep red cell suspension were incubated at 37°C for 10 min, centrifuged at 200 g for 5 min and left at room temperature for one hr. They were resuspended by agitation and examined for rosettes in a counting chamber.

Null-lymphocytes. When the proportion of cells showing spontaneous sheep cell rosettes (T-cells) and those showing the characteristics of B-cells were added together, the sum accounted for 57–91% in normal subjects. The remaining cells were termed null-cells.

Measurement of lymphocyte size. About 5×10^6 lymphocytes were resuspended in 5 ml RPMI and 10 ml Isoton (Coulter Electronics) and size measured in a Coulter Counter Model B using a 100 μ diameter orifice tube. The results were recorded on an automatic particle size distribution analyser, model J. The machine was calibrated with human

red cells whose MCV had been measured on a Coulter Counter model S.

Tests for humoral response

Escherichia coli antibodies. Antibodies in sera were looked for by an agglutination test with human red cells on to which *E. coli* antigens were absorbed (Webster, Effer and Asherson, 1974).

Pneumococcal polysaccharide type II antibodies. 1 mg of the antigen in 0.1 ml was given intradermally and response followed by titrating a pre- and post-injection (day 12) serum sample using a haemagglutination technique with antigen absorbed on to sheep cells (Barker, Stashak and Prescott, 1969; Gold and Fudenberg, 1967).

Haemophilus influenzae, E. coli, Staphylococcus aureus antibodies. Antibodies were looked for by injecting 0.1 ml of each antigen intradermally in the forearm. A control consisted of suspending fluid only. Results were read at 5 min, 4, 8 and 16 hr. Zone of induration, and erythema were measured.

Immunoglobulin levels. IgG, IgA and IgM levels were measured (Mancini, Carbonara and Herman, 1965).

Tests for cell-mediated response

Candida albicans and mumps. Antigens were injected intradermally (0.1 ml). A control was set up. The reaction was read at 48 hr. Purified protein derivative (PPD) (undiluted) was given by a multiple puncture method and read at 48 hr. 2,4 Dinitrochlorobenzene (DNCB) 0.1 ml as a sensitizing solution (0.5 g in 10 ml acetone) was applied to the inner aspect of the upper arm, covered with gauze and left for 7 days. After 7 days 0.1 ml of test solution (the above diluted 1 in a 1000) was applied to the other arm and read after 48 hr as either negative, discrete vesicle with induration, confluent vesicle, or induration, bullae, or ulceration.

Antigens. PPD was obtained from Evans Ltd; DNCB from Merck; mumps antigen from Eli Lilly; *E. coli*, *Staph. aureus* and *C. albicans* from Bencard; pneumococcal polysaccharide from Wellcome.

Results

B-, T- and null-cells (Table 3)

The percentage of B-cells in controls ranged from 5 to 23% (mean 12.1) and in CLL this ranged from 18 to 66% (mean 36.4). Expressed as absolute numbers 14 out of 15 patients had more than 2300 B-cells/ μ l which was the upper limit in controls. The range of B-cells was from 1620 to 68 000/ μ l. Unfortunately a total WBC count was not done on the control group and normal absolute numbers were based on a normal WBC range of 4000 to 1000 cells/ μ l.

TABLE 3. Spontaneous sheep-red-cell rosettes of T-lymphocytes, complement-receptor rosettes of B-lymphocytes and null-cells at first presentation plus one year later or after treatment in patients with CLL, lymphoma and controls

	Patients						Normal controls					
	At first presentation			Later/or after treatment								
	Total WBC	T-lymphocytes	B-lymphocytes	null-cells	T-lymphocytes	B-lymphocytes	null-cells	T-lymphocytes	B-lymphocytes	null-cells	T-lymphocytes	B-lymphocytes
		%										
*1	19.5	30	24	46	18	74	8	66	10	24	66	10
*2	10.4	22	37	41		Not done		68	7	25	68	7
*3	19.5	6	66	28		Not done		62	11	27	62	11
*4	27.1	6	24	70	4	61	35	48	23	29	48	23
5	46.1	1	30	69	49	53	0	74	8	18	74	8
*6	16.3	8	18	74	5	74	21	58	16	26	58	16
7	27.3	8	23	69	50	52	0	82	9	9	82	9
8	36.0	1	35	64	1	16	83	75	8	17	75	8
9	221.0	1	31	68	10	14	76	44	13	43	44	13
10	67.0	2	63	35	12	26	62	57	18	25	57	18
11	58.1	3	38	59	60	40	0	52	15	33	52	15
12	50.2	5	52	43	62	21	17	61	23	23	61	23
13	32.6	1	52	47	5	73	22	59	8	33	59	8
14	8.1	1	20	79		Died		52	9	39	52	9
15	30.8	0	33	67	9	24	67	69	12	19	69	12
16	16.5	0	2	98		Died		64	14	22	64	14
17	1.8	30	21	49	20	83	0	41	15	34	41	15
18	11.1	24	3	73		Died		78	5	17	78	5
Mean		6.3	36.4	57.7				61	12.1	25.7	61	12.1
±s.d.		8.6	15.2	15.6				11.6	4.6	8.5	11.6	4.6
s.e. mean		2.2	3.9	4.0				2.7	1.1	0.0	2.7	1.1

* Not treated. All others received treatment.

Null-cells were between 9 and 43% in controls (360–4300/ μ l) and were increased in numbers in all patients with CLL (4300–34 000/ μ l). The 3 patients with lymphoma had a normal number of B-cells, but 2 had an increased number of null-cells (8100 and 16 000/ μ l).

Eight of 15 patients with CLL had normal T-cell numbers and 7 had reduced numbers. These were low in one patient with lymphoma. Figure 1 shows this expressed as a % of total cells.

Nature of surface immunoglobulin

In 13 control subjects, cells reacting with anti-IgG, IgA and IgM constituted 7.9, 1.5 and 9.5% of the total respectively. The ratio of κ to λ light chains was 12 : 7 (1.7).

There was a significant rise in the proportion of cells carrying an IgM immunoglobulin in CLL (Table 4), IgG predominated in 3 patients and IgM in 4 patients. In addition, there was a marked disturbance in the light chain distribution. Three showed a preponderance of κ chains and 4 a preponderance of λ chains. The κ/λ ratio was abnormal in all but 2 patients with CLL, confirming the probably monoclonal origin of this disease (Fig. 2).

FIG. 1. Mean percentage of T, B, and null-cells before and after treatment. T = T-lymphocytes, B = B-lymphocytes.

Case 16 with lymphoma had lymphocytes with 98% of cells carrying IgM, and 97% of these cells had λ light chains.

TABLE 4. B-cell identification by surface staining in the patients before treatment
Per cent. of stained cells using the following antisera

Patient	Polyvalent	IgG	IgA	IgM	κ	λ	κ/λ ratio
1	27	17	3	10	10	24	0.4
2	32	25	4	10	32	10	3.2
3	47	8	0	33	18	34	0.5
4	74	67	5	23	14	72	0.2
5	88	12	6	56	80	18	4.4
6	37	3	0	36	10	25	0.4
7	46 (52)†	10 (13)	4 (6)†	33 (34)†	44 (40)†	13 (13)†	3.4
*8	46	2	5	35	45	0	0.0
9	91	82	0	19	6	92	0.07
*10	81 (88)†	12 (15)	5 (2)†	68 (80)†	0 (0)†	92 (90)†	0
*11	88 (91)†	12 (8)	3 (7)†	74 (78)†	20 (17)†	70 (75)†	0.29
*12	94	62	21	13	87	9	9.7
13	40	2	3	26	30	10	3.0
14	31	22	1	12	25	6	4.1
*15	100	0	3	95	95	5	19.0
Mean ($\pm$ s.e. mean)	61.5 (6.9)	22.4 (6.7)	4.2 (1.3)	36.2 (6.3)	34.4 (7.8)	30.8 (8.7)	
Range	27–100	0–82	0–21	10–95	0–95	0–92	
Patient							
16	100	7	5	98	20	97	0.2
*17	25 (100)†	4 (30)†	7 (4)†	20 (90)†	22 (100)†	10 (0)†	2.2
*18	44 (48)†	32 (36)†	4 (2)†	15 (12)†	28 (34)†	18 (15)†	1.6

* Cells treated with trypsin before staining.

† Figures are obtained on lymphocytes extracted from lymph gland.

Controls (13)

Mean ($\pm$ s.e. mean)	17.4 (1.3)	7.9 (1.4)	1.5 (0.4)	9.5 (0.9)	12.0 (0.9)	7.1 (0.5)	1.7
Range	10–25	2–18	0–4	5–17	8–18	3–11	1.1–3.3

Comparison of lymphocytes in blood and lymph node

In 3 patients with CLL and 2 with lymphoma lymphocytes from these 2 sources were compared. In the 3 patients with CLL identical results were obtained from blood and gland lymphocytes, as was the case with one patient with lymphoma.

In the second patient with lymphosarcoma, 90% of the lymphocytes from the gland carried IgM immunoglobulins and all had κ light chains. The blood lymphocytes were normal.

FIG. 2. Ratio of lymphocytes reacting with κ and λ antisera. $\circ$ Normal, $\bullet$ CLL, $\blacktriangle$ lymphoma blood, $\triangle$ lymphoma lymph gland.

Lymphocyte size (Fig. 3)

Cells in CLL tended to be larger than lymphocytes from normal subjects. In controls the cell diameters were mainly between 5.6 to 8.5 μ whereas in CLL the majority of lymphocytes were between 7.6 to 10.5 μ .

Cell-mediated immunity (Table 5)

All 9 controls became sensitized following application of DNCB whereas only 8 of 15 patients with CLL and one out of 3 lymphoma patients responded. A positive PPD test was found in about

FIG. 3. Size of lymphocytes assessed in Coulter Counter Model B in (a) normal (18) patients and (b) CLL (14) patients.

TABLE 5. Tests for cell-mediated immunity

	Number positive/number tested		
	Controls	CLL	Lymphoma
DNCB	9/9	8/15	1/3
PPD	4/10	8/15	0/3
<i>Candida</i>	9/10	9/15	1/3
Mumps	8/10	7/15	1/3

DNCB = dinitrochlorobenzene.

PPD = purified protein derivative (tuberculin).

50% of the controls and patients. Nine of 10 controls reacted to *Candida* and 8 of 10 to mumps antigen, whereas only 9 of 15 and 7 of 15 CLL patients responded.

The mildly affected patients who did not require treatment (cases 1 to 6) tended to respond positively whereas the patient who required treatment showed impaired responsiveness. Thus 75% of tests were positive in cases 1 to 6, whereas only 39% were positive in cases 7 to 15. In particular, the DNCB test was positive in cases 1 to 6 and only in cases 10 and 14 of the remaining patients.

Humoral immunity

Serum immunoglobulins. Serum IgG, IgA and IgM levels were reduced in 3, 5 and 6 out of 15 patients with CLL respectively. IgA was reduced in one patient with lymphoma and IgM reduced in all 3 lymphoma patients. The reduced IgG levels in the CLL patients were 2.7, 2.8 and 4.4 g/l (normal range 6–17 g/l), reduced IgA levels were 0.1, 0.2, 0.3, 0.3 and 0.4 g/l (normal range 0.5–2.2 g/l) and reduced IgM levels were <0.1, 0.2, 0.3, 0.3, 0.4 and 0.5 g/l (normal range 0.6–2 g/l). The reduced levels were found in the more severely affected patients. Three patients had low levels of all the immunoglobulins.

(13, 14 and 15) and 2 reduced IgA and IgM only (cases 10 and 11).

Response to pneumococcal polysaccharide type III. Thirteen control subjects showed a serum antibody titre 12–14 days after injection which ranged from 1/128 to 1/4096 (Fig. 4). Only 1 out of 10 patients with CLL reached a titre of 128 after sensitization. Two patients with lymphoma also showed a poor response. Treatment of the sera with 2-mercapto-ethanol showed that the antibody was both IgG and IgM.

FIG. 4. Rise in titre of pneumococcal polysaccharide antibody. Δ Lymphoma, $\circ$ CLL.

E. coli antibodies. Antibodies were found in all of 18 control sera tested, the lowest titre being 1 in 64. Eight of 15 patients with CLL had *E. coli* antibody titres of <64 as did 2 of 3 patients with lymphoma. Patients with CLL not requiring treatment had normal titres, and low titres were found in the more severely affected patients.

Antibody to Staph. aureus, H. influenzae and E. coli (intradermal tests). Positive results were obtained in 9 of 10 controls with *Staph. aureus*, in all 10 controls with *H. influenzae* and in 9 of 10 controls with *E. coli*.

Seven of 15 CLL patients gave positive results with *Staph. aureus*, 15 of 15 were positive with *H. influenzae* and 12 of 15 were positive with *E. coli*. Failure to respond occurred in the more severely affected patients, although one patient who did not require treatment (case 6) was negative to *E. coli*.

The effect of treatment

Tests were repeated in 13 out of 18 patients. Three died and the tests were not repeated in 2 mildly affected patients who did not require treatment.

Three other patients were retested not less than one year after the initial study although they were not treated. Nine treated patients with CLL were in 2 groups. Five patients (cases 5, 7, 11, 12 and 13) responded well to treatment with chlorambucil and 4 (cases 8, 9, 10 and 15) did not. Those who responded showed a marked decline in the proportion of null-cells, which disappeared in 2 patients (Table 3). In 3, the percentage of B-cells increased, remained unchanged in one and fell in a third. In all the percentage of T-cells rose.

In those failing to respond, the percentage of null-cells increased in 3 and was unchanged in the fourth. The percentage of B-cells and the percentage of T-cells increased with the fall in total lymphocyte numbers.

In the patient with lymphosarcoma the null-cells disappeared from the blood after treatment and the percentage of B-cells increased. Results are summarized in Fig. 1.

In cases 5 and 7, the satisfactory response was accompanied by decline in the numbers of lymphocytes with IgM and κ markers (Table 6). In case 8, who did not respond, the IgM-producing cells increased.

TABLE 6. Comparison between lymphocytes carrying surface receptors before (a) and after (b) treatment

Patient	Percentage of stained cells using the following antisera					
	Polyvalent	IgG	IgA	IgM	κ	λ
5a	88	12	6	56	80	18
5b	29	8	6	8	24	12
7a	46	10	4	33	44	13
7b	24	13	5	9	21	5
8a	46	2	5	35	45	0
8b	100	44	5	68	72	30
17a	25	4	7	20	22	10
17b	16	6	0	13	14	3

Re-testing of 3 patients with CLL who were not treated (cases 1, 4 and 6) showed an increase in B-cells at the expense of null-cells. The PPD and DNCB skin tests were repeated as described either after treatment or in the untreated patients after one year. In 3 patients with CLL the results remained unchanged. Tests in 8 patients with CLL repeated after treatment are summarized in Table 7.

TABLE 7. Effect of treatment on cell-mediated immunity in chronic lymphocytic leukaemia

	Number positive/number tested	
	Before treatment	After treatment
PPD	4/8	3*/8
DNCB	2/8	1*/8

* Including one patient who was initially negative.

One patient received treatment with prednisone followed by chlorambucil and subsequently by cyclophosphamide over a 2-year period. She was reasonably well but the total lymphocyte count was always approximately 40 000/ μ l. Initially she gave negative results when tested with PPD and DNCB. On re-testing 7 weeks after stopping treatment she gave a very strongly positive response to PPD and an alarming response to DNCB with massive weal formation and oedema of the arm. Prednisone 60 mg/day was started after 24 hr and oedema subsided leaving an ulcerated area which healed normally.

Six other patients had good clinical responses to chlorambucil therapy, in some preceded by prednisone therapy. These patients were retested in remission 6 or more weeks after cessation of therapy. In 4 of these patients there was no change in the way they reacted to PPD and DNCB. In one patient receiving chlorambucil alone and who had a good clinical response, a positive response to DNCB was followed by a negative one in remission. A second patient who responded to a combination of prednisone and chlorambucil lost his initially positive response to PPD.

There was no change in immunoglobulin levels after treatment.

Thus, following treatment the patients' immune responses were either unchanged or continued to decline.

One patient with lymphosarcoma was treated with 6 courses of MOPP at 2-monthly intervals. He initially gave a negative PPD and a strongly positive DNCB test. The latter became negative after treatment.

Discussion

The observations reviewed in the introduction concerning the B-cell origin of CLL have been confirmed in this study, almost all the patients showing increased numbers of B-lymphocytes. In addition, there were, as noted by others, lymphocytes which had surface immunoglobulin but lacked complement receptors (Seligmann, 1974). Furthermore, cells which react with heat-aggregated immunoglobulin while failing to react with an anti-IgG serum do occur (Davis, 1976). These cells are similar to the cells termed null-cells in this paper. A new observation is the great susceptibility of these null-cells to the effects of antileukaemic agents. In those patients who responded to drugs such as chlorambucil, the fall in lymphocyte numbers was due mainly to the considerable fall in the numbers of null-cells. However, the relative proportion of B and null-cells may vary at different stages of the disease, without treatment, as shown in the untreated cases as well as by Davis (1976).

The clonal origin of CLL recently reviewed by Fialkow (1976) and Greaves (1975) is confirmed in these studies by the preponderance of either a κ or λ light chain on the cell surface.

Comparison of cells in peripheral blood and lymph gland shows that in CLL the cells were identical. In one patient with lymphocytic lymphoma gland cells were all B lymphocytes with κ light chains, whereas lymphocytes from peripheral blood were normal, indicating the localized origin of this clonal disease.

Cells in CLL tend to be larger than normal lymphocytes but this does not apply to all the patients.

The failure of immune responses and the associated hypogammaglobulinaemia is well known (see introduction). In all the tests the patients with CLL showed significant impairment as compared to controls and humoral immunity was more severely impaired than cell-mediated immunity. The most satisfactory test was the response to a challenge of pneumococcal polysaccharide which was impaired in 14 out of 15 patients with CLL. It was abnormal even in those patients who were in good health or whose only manifestation was an elevated lymphocytic count.

Finally, the effect of treatment in those who responded was to reduce the number of lymphocytes, and hence reduce lymphadenopathy and size of infiltrated organs. Removal of lymphocytes from marrow may have led to a rise in the red cell count, neutrophils and platelets. Their immune capacity, however, was not benefited by treatment but at the best remained unchanged or continued to decline. This is in contrast with acute adult leukaemia where improvement in immune status indicated a likely response to chemotherapy (Hersh *et al.*, 1971).

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