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# Two Distinctive Lymphocyte Populations in Chronic Lymphocytic Leukaemia

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**Abstract**—*The peripheral blood and marrow lymphocytes from 20 patients with chronic lymphocytic leukaemia (CLL) were studied. Two populations of lymphocytes were identified on the basis of size, kinetics and deoxyribonucleic acid (DNA) content. These were present at various stages of the illness but did not correlate with any clinical findings or prognosis.*

## INTRODUCTION

THE PRESENTATION and course of CLL is so varied that it is often wise, initially, to observe the patient for some time without treatment to appreciate the rate of progression of the individual's illness. This diversity has encouraged research workers to look for features which might indicate the likely prognosis. Such studies have, until recently, been based on relating lymphocyte morphology to the clinical findings [1–4].

The majority of CLL lymphocytes have been described as small cells with scanty cytoplasm, dense nuclear heterochromatin and inconspicuous or absent nucleoli, [4]. However, in each patient there exists a number of larger cells with more cytoplasm, open nuclei and definite nucleoli. Some cells resemble prolymphocytes [5, 6] while others, particularly at the terminal stage, may become more akin to lymphosarcoma cells or blast cells [2, 3, 4].

A significant correlation between cell size and the survival time in CLL was shown by Gray *et al.* [7]. Those patients whose cells were mainly small, lived for $8\frac{1}{2}$yr compared to $2\frac{1}{2}$yr for patients whose peripheral blood contained many large cells. The degree of bone marrow infiltration was inversely related to the longevity of the patient.

The prognosis of CLL has been correlated to clinical staging of the disease [8]. Rai *et al.* showed that patients with stage 0, i.e., bone marrow and blood lymphocytosis only, had a median survival time of 150 months. Those patients in stage I (lymphocytosis with enlarged nodes), stage II (lymphocytosis with enlarged liver or spleen or both), stage III (lymphocytosis with anaemia) and stage IV (lymphocytosis with thrombocytopenia) had median survival times of 101, 71, 19 and 19 months respectively.

Recently other lymphocyte markers have become available and these have been used by Han *et al.* [9] to study the relationship between sheep erythrocyte rosette forming cells (SERFC), erythrocyte antigen complement rosette forming cells (EACRFC), and the course of the disease, but the results show no clear correlation. Further work is necessary to establish which are the crucial parameters in determining the course of the illness. There is evidence that the population of lymphocytes in the bone marrow is not the same as that in the peripheral blood [10]. Despite this, marrow lymphocyte kinetics have not been used in any assessment of the disease process.

The present work was undertaken to establish whether a relationship existed between the numbers of large and small lymphocytes, the proportion of SERFC, EACRFC and Mouse erythrocyte rosette forming cells (MERFC), and the prognosis in CLL. An attempt was made to correlate the kinetic behaviour of the large and small lymphocytes with the course of the leukaemia.

## MATERIALS AND METHODS

*Patients*

Twenty patients with CLL were studied

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during the course of their illness and classified on the basis of Rai [8]. Seven patients were in stage 0, one in I, five in II, five in III and two in IV (MRC CLL trial in progress). The diagnosis of CLL in patients with absolute lymphocyte counts of $<10{,}000 \times 10^9/l$ in the peripheral blood, or with $<40\%$ lymphoid cells in the bone marrow was based on the morphological appearance of lymphocytes, and the presence of MERFC and smear cells in both peripheral blood and bone marrow. All patients had $>10\%$ MERFC at some stage during the course of the disease. Repeat peripheral blood and bone marrow studies (MRC CLL Trial No. 1) confirmed the diagnosis of CLL. Peripheral blood, and bone marrow samples, were collected during routine clinical visits, or when indicated i.e., sudden enlargement of spleen, liver or nodes, or a marked change in the haematological findings.

Some patients in stage II, and those in stage III, IV were treated with chlorambucil and prednisone.

*Measurement of cell size*

The percentage of large and small cells in each sample was estimated using a micrometer on a Jenner Giemsa stained film. In order to establish that the populations of circulating lymphocytes (large and small) remained stable during the period of incubation the proportion of each type of cell was determined on a stained film, before and after 1 hr of incubation at 37°C.

*Table* 1. *Effect of incubation at* 37°C *on the percentage of large cells in the peripheral blood of patients with CLL*

| Large cells (%) | |
|---|---|
| Before 1 hr | After 1 hr |
| 2.4 | 2.1 |
| 5.6 | 8.3 |
| 12.0 | 10.0 |
| 10.0 | 10.0 |

*Radioactive labelling*

Peripheral blood and bone marrow samples were collected into bottles containing potassium EDTA. Aliquots of each sample were incubated at 37°C with 2.5 $\mu$Ci/ml of $^3$H-TdR (specific activity 5 Ci/mmole) and 5 $\mu$Ci/ml of $^3$H-UR (specific activity 5 Ci/mmole) for 30 and 60 min respectively [11]. Thin smears were made from the buffy layer and air dried. On each of these slides a smear from the buffy layer of a normal individual was spread for use as an internal control.

*DNA measurement*

The DNA content of CLL cells and control cells was measured on Feulgen stained preparations [12] using an M85 Vickers Microdensitometer fitted with a $\times 100$ oil immersion objective, (Vickers Instruments Limited, York). A rapid method was used requiring only one scan with a background setting. Fifty large CLL cells, fifty small CLL cells, and twenty control cells were measured using an appropriate mask size, chosen by visual observation, not by calculation, so as to be large enough to cover the whole cell, yet small enough not to include too much background. Results obtained were in arbitrary integrated optical density units. The inherent experimental error of the method was taken into account by spreading a control smear on each slide, so that comparative measurements were made on the same slide as it had been noted by Mayall [13] that a highly significant variation in stain intensity occurs from slide to slide with Feulgen preparations.

*Cell surface markers*

The percentages of SERFC, EACRFC and MERFC were estimated. Heparinized blood was layered onto Lymphoprep and centrifuged at 400 ***g*** for 15 min. The lymphocytes obtained at the interface were adjusted to a count of $4 \times 10^6$/ml. SERFC and MERFC were made by mixing 0.25 ml of lymphocyte suspension with 0.05 ml foetal calf serum and 0.25 ml of appropriate red cell suspension, i.e., papainized sheep red cells for SERFC, and mouse red cells for MERFC. The tubes were centrifuged at 114 ***g*** for 2 min and incubated on ice at 4°C for 1 hr. The cells were gently suspended and counted. EACRFC were formed by mixing 0.5 ml of lymphocyte suspension ($2 \times 10^6$/ml) with 0.5 ml sheep cells sensitized with rabbit haemolytic serum with complement added and incubated at 37°C for 5 min, centrifuged at 114 ***g*** for 2 min and left at R.T. for 30 min. The cells were vigorously resuspended and counted [14]. Any lymphocyte with three or more adhering red cells was considered a rosette. In examining 10 different peripheral blood films, the number of monocytes was less that 1/1000 and therefore it was considered that the rosetting technique

was adequate for distinguishing B cells from others.

## RESULTS

*Lymphocyte populations*

In the peripheral blood and bone marrow, two populations of lymphocytes, based on cell size, were observed. The small lymphocytes measured $<8$ μm in diameter, had dense chromatin and scanty cytoplasm, whilst the large lymphocytes measured 8–15 μm and showed a finer chromatin appearance with more cytoplasm and often definite nucleoli.

The proportion of large cells in both peripheral blood and corresponding bone marrow was found to be similar in 7 patients. In only 1 case were there three times as many large lymphocytes in the marrow as in the peripheral blood.

In 13 peripheral blood specimens from 9 untreated patients, the number of large cells varied from 5 to 34% (mean 11.6%). A similar variation was seen in 13 peripheral blood samples from 5 patients who had received chemotherapy i.e., 1–40% (mean 11.9%).

In the bone marrows examined from 6 untreated patients the number of large cells varied from 6 to 24% (mean 11%). Similarly the number of large cells in the bone marrows from 3 treated patients ranged from 9 to 20% (mean 14%).

*Table 2. Percentage of large cells in the bone marrow and peripheral blood of treated and untreated patients with CLL*

| | No. of specimens | Mean % | Range |
|---|---|---|---|
| Peripheral blood Treated | 13 | 11.9 | 1–40 |
| Peripheral blood Untreated | 13 | 11.6 | 5–34 |
| Bone marrow Treated | 3 | 14.6 | 9–20 |
| Bone marrow Untreated | 6 | 11.0 | 6–24 |

Serial examination of peripheral blood from 5 patients throughout the course of their disease, showed that the proportion of large cells varied considerably from one estimation to the next and there were times when only small cells were present.

*Rosettes*

The percentage of SERFC, EACRFC and MERFC varied during the course of the disease. Table 3 shows the percentage of different types of cells compared to the corresponding number of large lymphocytes in the peripheral blood. There was no correlation between the percentage of large lymphocytes and any of these markers.

*Labelling index (LI)*

*Peripheral blood.* A total number of 90 specimens were examined from 20 patients. The LI with $^3$H-TdR was less than 1% in 88 specimens. Cells in mitosis were not present. In patient 5 labelling indices of 2.5 and 4% were obtained when the disease course became aggressive.

The number of cells synthesizing RNA in 16 samples from 12 patients varied from 32 to 95% with the mean of 72%. In all specimens examined, the intensity of labelling as measured by grain count was similar in both small and large cells. In order to establish whether the *in vitro* environment enhanced the cell death, peripheral blood samples were divided into two aliquots. $^3$H-UR was added immediately to the first sample whilst the other was incubated for 1 hr before the addition of the isotope. There was no difference in the proportion of labelled cells in these specimens.

*Bone marrow.* In each of 9 specimens the number of lymphoid cells incorporating $^3$H-TdR was $<1\%$. In one instance the large cells had a LI of 2.5% (patient No. 4), but the LI of the small cells was still $<1\%$. Three specimens labelled with $^3$H-UR gave uptake values between 41 and 97% of the lymphocytes.

*Measurement of nuclear DNA content*

*Normal cells.* The DNA content of small lymphocytes, large lymphocytes, monocytes and polymorphonuclear neutrophils, was measured on Feulgen stained preparations from seven normal individuals. There was no significant difference between the DNA content in these cells, the amount varying by only 0.01–3.62 arbitrary units within cell types. All three distributions showed a relatively small scatter suggesting a high degree of consistency in these normal cells. In Fig. 1 the nuclear DNA content of both large lymphocytes and monocytes and small lymphocytes are shown.

*Table 3. A comparison of the percentage of large cells in the peripheral blood, with SERFC, EACRFC and MERFC in patients with CLL.*

| Patient | SERFC | EACRFC | MERFC | Large lymphocytes |
|---|---|---|---|---|
| 2 | 1 | 2 | 1 | 9.0 |
| | 15 | 12 | 33 | 19.0 |
| | 12 | 6 | 11 | 1.0 |
| 3 | 5 | 8 | 15 | 22.0 |
| | 14 | 10 | 25 | 34.0 |
| | 1 | 4 | 53 | 7.4 |
| | 11 | 10 | 41 | 19.0 |
| 4 | 6 | 7 | 16 | 13.0 |
| | 8 | 9 | 61 | 1.5 |
| | 5 | 9 | 50 | 1.0 |
| | 5 | 10 | 53 | 10.0 |
| | 20 | 14 | 28 | 10.0 |
| 5 | 5 | 2 | 5 | 20.0 |
| 6 | 19 | 12 | 31 | 9.0 |
| 7 | 73 | 42 | 16 | 8.5 |
| 8 | 1 | 3 | 67 | 4.9 |
| | 2 | 1 | 52 | 5.4 |
| 9 | 17 | 24 | 37 | 9.0 |

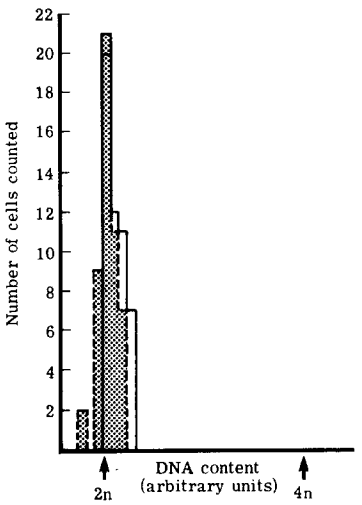


*Fig. 1. Nuclear DNA content (arbitrary units) of both large and small monoclear cells in normal peripheral blood.*
▨ *large lymphocytes plus monocytes.*
□ *small lymphocytes.*

*Leukaemic cells*

*Peripheral blood.* Table 4 shows the mean nuclear DNA content of both small and large cells in the peripheral blood of patients with CLL, compared to the control cells. A double population of cells was noted in 13 samples while nine specimens revealed a single population only. Both single and double populations were observed in individual patients at some stage during the disease. The mean DNA values of both the large and small CLL lymphocytes in the majority of specimens examined was greater than that of the control cells.

*Table 4. Mean DNA values (arbitrary units) of large and small lymphocytes in peripheral blood samples from patients with CLL*

| Patient No. | Large lymphocytes | | | Small lymphocytes | | | Control cells | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | S.D. | Range | Mean | S.D. | Range | Mean | S.D. | Range | Population |
| 1 | 83.47 | 3.95 | 76–92 | 80.45 | 3.81 | 72–88 | 70.0 | 2.83 | 66–74 | Single |
| 2 | 111.45 | 9.64 | 88–126 | 94.84 | 4.70 | 82–106 | 90.2 | 6.29 | 80–100 | Double |
| | 132.16 | 6.99 | 120–144 | 123.44 | 3.16 | 116–134 | 111.65 | 3.84 | 106–116 | Double |
| | No large cells | | | 93.96 | 2.23 | 90–98 | 88.90 | 5.65 | 76–98 | Single |
| 3 | 133.88 | 5.66 | 116–144 | 108.90 | 5.85 | 96–120 | 102.55 | 5.14 | 96–108 | Double |
| | 121.32 | 2.99 | 116–126 | 111.60 | 2.21 | 106–116 | 99.33 | 2.22 | 94–104 | Double |
| | 90.00 | 5.60 | 78–98 | 84.08 | 3.83 | 78–92 | 84.73 | 2.05 | 82–90 | Double |
| | 80.16 | 5.61 | 70–94 | 84.32 | 4.51 | 78–94 | 75.60 | 4.32 | 70–82 | Single |
| 4 | 50.01 | 7.42 | 36–64 | 43.00 | 3.56 | 34–48 | 36.61 | 2.74 | 32–44 | Double |
| | 118.08 | 4.14 | 104–124 | 99.92 | 8.10 | 80–116 | 94.91 | 2.88 | 90–100 | Double |
| | No large cells | | | 114.26 | 1.99 | 110–122 | 108.57 | 2.99 | 104–112 | Single |
| | 95.43 | 3.16 | 88–102 | 86.63 | 2.39 | 82–92 | 82.58 | 4.20 | 76–90 | Double |
| | 94.48 | 5.58 | 82–102 | 90.64 | 4.15 | 78–98 | 82.89 | 4.46 | 74–90 | Single |
| 5 | 87.76 | 2.51 | 82–92 | 88.60 | 3.48 | 80–94 | 85.11 | 2.57 | 80–90 | Single |
| | 75.53 | 5.29 | 66–84 | 80.52 | 1.86 | 74–84 | 77.24 | 3.09 | 74–84 | Single |
| 6 | 86.37 | 6.68 | 76–100 | 95.10 | 4.78 | 84–104 | 94.67 | 3.07 | 88–100 | Double |
| 7 | 102.37 | 7.16 | 86–122 | 90.38 | 4.14 | 80–96 | 95.52 | 2.66 | 90–100 | Double |
| 8 | 100.84 | 3.66 | 90–110 | 100.72 | 2.24 | 94–104 | 90.82 | 3.75 | 82–96 | Single |
| | 88.32 | 4.28 | 78–98 | 82.24 | 3.79 | 70–90 | 77.08 | 3.97 | 70–82 | Double |
| 9 | 92.35 | 4.39 | 82–98 | 74.64 | 3.37 | 66–78 | 76.92 | 3.84 | 70–81 | Double |
| 10 | No large cells | | | 78.04 | 8.46 | 54–94 | 77.11 | 2.59 | 74–82 | Single |
| 11 | 107.32 | 3.25 | 102–114 | 103.16 | 4.54 | 88–112 | 102.58 | 4.59 | 94–110 | Double |

*Bone marrow.* In Table 5, the mean nuclear DNA content of small and large cells in the bone marrow together with those of controls are shown. The DNA content of both large and small cells was consistently higher than the controls (whether normal lymphocytes or patient's own segmented polymorphs were used). Figure 2 demonstrates histograms of 3 bone marrow samples showing clearly the picture of both double and single populations, and also the presence of not only a double population but a spread of large cells around $G_1$ with many cells between $G_1$ and $G_2$ and some over 4n.

*Results in individual patients*

Patient 1 showed a distinct double population both in peripheral blood and bone marrow 1–2 weeks after chemotherapy.

Patient 2 had many atypical cells in both peripheral blood and bone marrow and a double population at diagnosis. He was treated with an intermittent course of prednisone and chlorambucil to which the response was very good. The double population persisted during the first course of chemotherapy but during the second course only one population was present (Fig. 3).

In patient 3 the clinical and haematological condition remained static for 3 yr. During this time there were changes in the proportions of large and small lymphocytes. A single population was found in the peripheral blood while two populations were present in the bone marrow. Later a very distinct double population was seen in the peripheral blood which became less marked in the subsequent investigations. The leukaemia then became more active and she developed generalised lymphadenopathy with a rise in white blood cell count. She was treated with chlorambucil. A double population persisted for a week after chemotherapy but disappeared at the end of the treatment.

In patient 4 a significant double population was noted in both bone marrow and per-

*Table 5. Mean DNA values (arbitrary units) of large and small lymphocytes in bone marrow samples from patients with CLL*

| Patient No. | Large lymphocytes Mean | S.D. | Range | Small lymphocytes Mean | S.D. | Range | Control cells Mean | S.D. | Range | Population |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 123.25 | 6.16 | 108–136 | 111.84 | 7.14 | 96–128 | 93.20 | 10.02 | 80–114 | Double |
| 2 | 102.53 | 3.99 | 90–112 | 96.08 | 3.19 | 90–104 | 86.8 | 4.26 | 78–92 | Double |
| 3 | 82.42 | 5.12 | 70–90 | 77.11 | 5.83 | 62–84 | 74.45 | 7.07 | 61–83 | Double |
| 4 | 72.68 | 16.22 | 42–94 | 45.98 | 5.27 | 32–52 | 42.44 | 3.58 | 36–50 | Double |
| 4 | 128.56 | 3.45 | 119–137 | 100.58 | 4.79 | 91–108 | 116.62 | 7.93 | 100–126 | Double |
| 5 | 116.78 | 8.35 | 96–132 | 118.88 | 5.82 | 104–140 | 97.00 | 4.90 | 90–104 | Single |
| 11 | 107.27 | 3.23 | 102–112 | 101.72 | 3.31 | 88–108 | 98.58 | 4.59 | 90–106 | Double |
| 12 | 88.69 | 6.74 | 70–98 | 83.45 | 5.44 | 72–92 | | | | Double |
| 13 | 63.66 | 2.75 | 56–68 | 64.35 | 2.24 | 58–68 | 60.86 | 2.13 | 55–65 | Single |

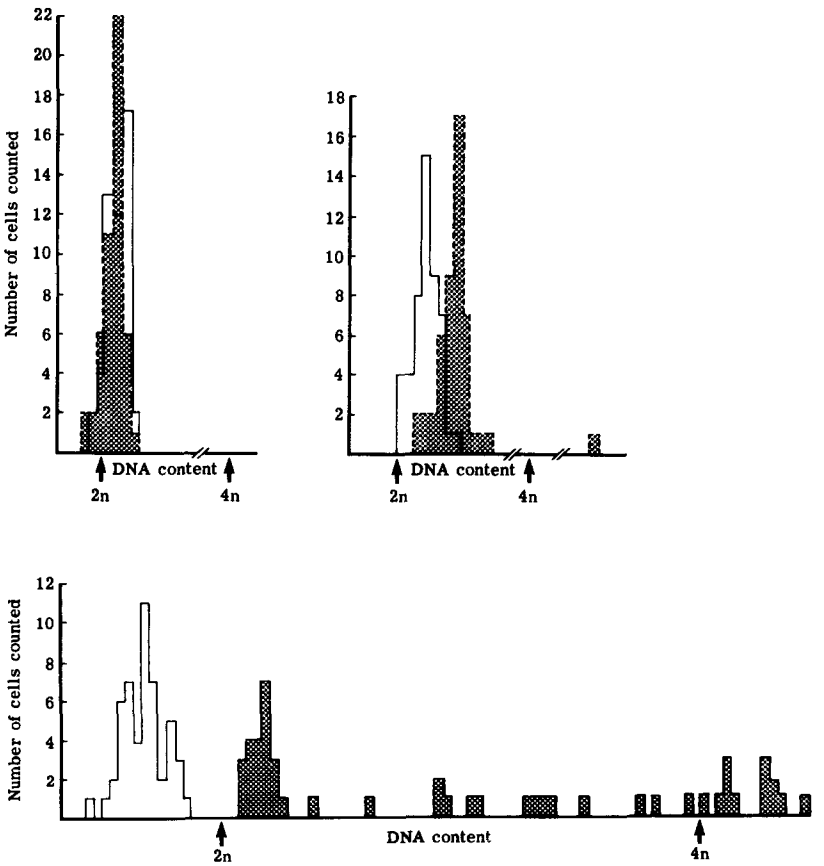


*Fig. 2. Three representative histograms of the nuclear DNA content (arbitrary units) of large and small lymphocytes in the bone marrow of patients with CLL.*
▨ *large lymphocytes.*
□ *small lymphocytes.*

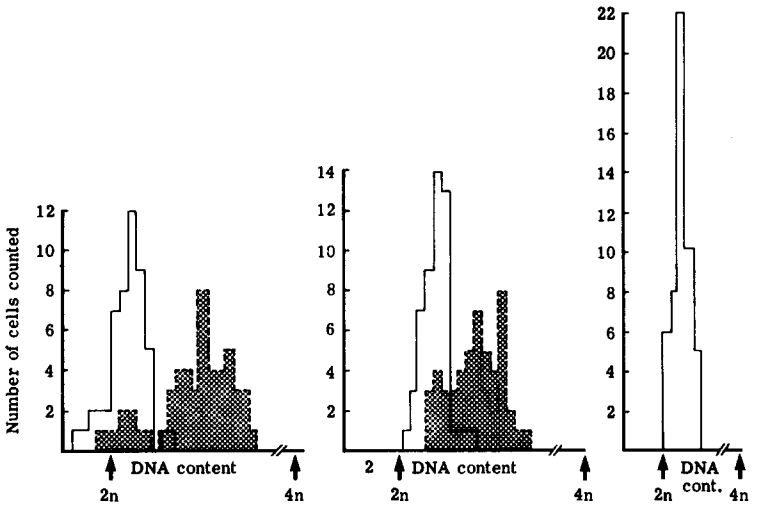


*Fig. 3. Nuclear DNA content (arbitrary units) of large and small lymphocytes in the peripheral blood of Patient 2 during the course of the leukaemia.*
▨ *large lymphocytes.*
□ *small lymphocytes.*

ipheral blood at diagnosis. The disease remained in a steady-state until 17 months later when the patient developed generalized lymphadenopathy, hepatosplenomegaly and rise in lymphocyte count. The peripheral blood at this time showed a single population. Therapy with chlorambucil for 4 weeks caused an improvement in the general condition and reduction in the white cell count. The study of nuclear DNA content in the circulating lymphocytes two weeks after chemotherapy showed a double population and a spread of cells around $G_1$ but there were no cells in $G_2$ or above 4n. The corresponding bone marrow showed not only a definite double population but also a spread of cells around $G_1$ with many between $G_1$ and $G_2$ and some over 4n. The presence of some cells with a DNA content between 2 and 4n should indicate a population in S. However, the LI of these cells was only 2.5%. The chromosome studies were unsuccessful. It is therefore not possible to consider that the population of large cells was heterogenous for chromosome number, but it may be that these cells are arrested in S and perhaps the presence of a surface protein or an antibody antigen complex complex has prevented the cells from taking up $^3$H-TdR. A single population was noted again in the specimen studied 14 weeks following cessation of chemotherapy and a repeat sample two weeks later showed a double population. No significant change was present in either the total lymphocyte count or the general condition of the patient at this time.

Patient 5 developed aggressive disease with a marked lymphadenopathy and hepatosplenomegaly and a submandibular mass. The lymphocyte count rose sharply towards the final stages of leukaemia. The proportion of large cells increased to 40% with a marked reduction in the number of smear cells. The LI of large cells with $^3$H-TdR rose to 2.5% and then 4%.

*Effect of chemotherapy*

Two lymphocyte populations were present in 8 out of 12 patients, at diagnosis or before chemotherapy had commenced. Shortly after treatment (1–2 weeks) two populations of lymphocytes appeared even when there had previously been only one recognisable, but some time later a single population reappeared.

The DNA content of lymphocytes from untreated patients showed that the majority of cells were in the $G_0/G_1$ stage. This was also true for patients following chemotherapy except for one, whose cells showed a definite population between $G_1$ and $G_2$ with few cells in S. The LI of lymphocytes in this patient was only 2.5% which indicates that somehow the cells failed to incorporate $^3$H-TdR.

In Table 6 the lack of correlation between lymphocyte populations, lymphadenopathy, hepatosplenomegaly and chemotherapy are shown. It was not possible to correlate the appearance of a single or double population with the course of the disease.

*Table 6. Relationship between hepatosplenomegaly, lymphadenopathy and chemotherapy, with single and double populations found in the peripheral blood of patients with CLL*

| Lymphocytes | Spleen ↑ | Spleen N | Liver ↑ | Liver N | Nodes ↑ | Nodes N | Chemotherapy On | Chemotherapy Off |
|---|---|---|---|---|---|---|---|---|
| Double population | 8 | 7 | 7 | 8 | 6 | 9 | 4 | 11 |
| Single population | 7 | 3 | 7 | 3 | 4 | 6 | 3 | 8 |

## DISCUSSION

The kinetic behaviour of lymphocytes in CLL has not been the subject of much study in the past, probably due to the generally held belief that lymphocytes in CLL are mature quiescent cells. The labelling of lymphocytes with $^3$H-TdR has shown that, on the whole, less than 1% of cells in the bone marrow and peripheral blood are capable of DNA synthesis. The apparent longevity of the lymphocytes [15] is in agreement with the prolonged $G_1$ of these cells. In two patients Theml [15] gave a continuous infusion of $^3$H-TdR for 7 days. The LI of small lymphocytes changed very little and remained 3% in both bone marrow and peripheral blood. The LI of large cells being 3.5% preinfusion, rose markedly to 41% in bone marrow and 62% in peripheral blood by the 7th day. Thus the turnover of the small cells was prolonged and likely to be about one year whilst the large cells probably had a generation time of 7–14 days. Two of our patients with progressive disease had many large lymphocytes and these cells had Labelling Indices of 2.5 and 4%. A large number of lymphocytes in our CLL patients had RNA synthetic activity. This has been noted by others in this condition and in acute lymphoblastic leukaemic (ALL) [16–19]. Hyman [18] found a high degree of labelling (65–75%) in both active and remission states of CLL, although the grain counts were lower in the latter.

No reference was made regarding the large and small lymphocytes in the bone marrow or the peripheral blood but our findings indicate a similar grain count in the two populations, suggesting a uniform abnormality of RNA metabolism.

In the majority of our patients the mean DNA content of both bone marrow and peripheral blood lymphocytes in $G_1$ and $G_0$ was higher than controls on the same slide. This was true for both large and small cells although the former had the highest mean DNA value. Gahrton and Foley [20] demonstrated that in two cases of CLL the basic Feulgen DNA values were about 10% greater than in normal lymphocytes. In one case a small percentage of cells were characterised by large mass values. Increased mass values have also noted previously, [21] in lymphocytes derived from patients with CLL as compared to normal lymphocytes. In our studies comparative measurements were always performed on cells on the same slide to avoid the variation in staining intensity which occurs from slide to slide with Feulgen preparations [13]. Our methods demonstrate relative quantitative values by making comparative studies which are more reliable and reveal more information than attempts to obtain absolute measurements.

The reason for the higher DNA value is not clear. Cytogenetic studies are generally unhelpful in CLL and we have been unable to demonstrate chromosome changes (personal observations). It is possible that the DNA is not being expressed correctly as measured by the Feulgen stain, and that there is a difference in the rate and extent of hydrolysis between the normal and leukaemic cells [22, 23].

There was a highly significant difference ($P = < 0.0005$) in the DNA content of the small and large cells. It is very unlikely that the large cells were in early S as would be the case in normal haemopoietic tissue [24] because the LI was so low, usually $< 1\%$. It is more likely that the two cell populations have an independent cell cycle but are both in the $G_0$–$G_1$ stage.

We found no correlation between the number of large cells and the percentages of SERFC, EACRFC and MERFC in eight patients with CLL. These markers, although useful for diagnosis, do not appear to relate to any of the other parameters we have studied e.g. DNA content and LI with $^3$H-TdR and $^3$H-UR. Sabolović *et al.* [25] found no cor-

relation with cell surface markers and three distinct subgroups of CLL as examined by electrophoretic mobility.

Our studies showed that the number of circulating large cells varies greatly from one patient to the next and also during the course of the disease. We were unable to relate the proportion of large cells to prognosis. Two populations were observed in most of our patients at one time or another regardless of the state of the leukaemia. In one patient we found a significant number of large cells associated with progressive disease. Basu *et al.* [26] reported a similar finding in two patients with CLL. However, his patients showed characteristics of lymphosarcoma rather than CLL.

There is still no clear indication as to the origin and significance of the two lymphocyte forms. We suggested that a circulating globulin might attach to lymphocytes causing their longevity and interfering with their function [27].

The failure to show any degree of proliferative activity in the bone marrow or peripheral blood raises the possibility that the large lymphocytes originate outside the circulation.

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