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Separation of T Lymphocytes from Normal Individuals and Patients with B Lymphocyte Chronic Lymphocytic Leukaemia

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Summary. Previous studies have shown that lymphocytes from patients with chronic lymphocytic leukaemia have a diminished response to mitogens which stimulate T cells. Chronic lymphocytic leukaemia is most often a disease of accumulating B cells so that T lymphocytes are diluted by large numbers of leukaemic cells. Direct comparison with the responses of normal lymphocytes to mitogenic stimulation is therefore suspect. To circumvent this difficulty, a method of isolating T cells from normal individuals and patients with chronic lymphocytic leukaemia was developed. Lymphocytes containing an average of 16·1 per cent B cells from normal individuals were applied to IgG–anti-IgG-coated Degalan bead columns and held at 4° for 2 hours. The eluted cells contained less than 2 per cent B cells. When chronic lymphocytic leukaemic lymphocytes, containing an average of 68·6 per cent B cells, were applied to IgG–anti-IgG columns, the eluted cells contained 36·4 per cent B cells. To improve the purification of T lymphocytes, columns of uncoated Degalan beads were used to remove non-specifically adherent cells. Eluted lymphocytes were then applied to IgG–anti-IgG columns. This resulted in the recovery of purified populations of T cells with less than 2 per cent contamination with B cells.

Patients with chronic lymphocytic leukaemia were found to have lymphocytes with either a normal density or a low density of surface immunoglobulins. B cells were successfully removed from lymphocyte suspensions in all cases of chronic lymphocytic leukaemia with a normal density of lymphocyte surface immunoglobulins. In the three cases of chronic lymphocytic leukaemia with low density surface immunoglobulins, separation by this method was unsuccessful. However, an enriched T-cell population was obtained when leukaemic lymphocytes which had lost all detectable surface immunoglobulins were passed through a column coated with heat-aggregated IgG.

INTRODUCTION

Several studies suggest that with rare exceptions, the leukaemic cells in chronic lymphocytic leukaemia are B lymphocytes with demonstrable surface immunoglobulins

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(Grey, Rabellino and Pirofsky, 1971; Wilson and Nossal, 1971; Preud'homme and Seligmann, 1972; Shevach, Herberman, Frank and Green, 1972; Aisenberg and Block, 1972; Ross, Rabellino, Polley and Grey, 1973). Study of the functional integrity of the T lymphocytes in this condition is hampered because they are diluted by large numbers of leukaemic B lymphocytes. T lymphocytes are important in cellular immune responses often elicited by viruses, certain other micro-organisms, and tumour-related antigens (Hellström, Hellström, Pierce and Yang, 1968; Dan, Kulcśar, Sallay and Naśz, 1971; Wybran and Fudenberg, 1973). Patients with chronic lymphocytic leukaemia may have impaired cellular immune responsiveness (Miller, Lizardo and Snyderman, 1961; Sokal and Primikirios, 1961) and an increased incidence of other cancers (Miller, 1962). It is not clear if this is due to impaired T-lymphocyte function, reduction in their absolute numbers, or some overriding effect of the large number of B lymphocytes. In order to study T lymphocyte function *in vitro*, it was felt necessary to separate the leukaemic B cells from the smaller number of T cells. This report describes a technique which was successful in obtaining a purified population of viable T lymphocytes from the peripheral blood of normal and leukaemic subjects and provides further information on the characteristics of the B and T lymphocytes in chronic lymphocytic leukaemia.

MATERIALS AND METHODS

Patients

Twenty-seven normal individuals and ten patients with chronic lymphocytic leukaemia were studied. The diagnosis of chronic lymphocytic leukaemia was established using generally accepted criteria (Levin, 1968).

Recovery of lymphocytes from peripheral blood

Twenty millilitres of venous blood were collected in heparinized tubes and allowed to sediment at 37° for 30 minutes. The supernatant plasma was removed, the tubes were centrifuged at 1000 ***g*** for 10 minutes and the buffy coat cells were added to the supernatant plasma. Appropriate blood group antiserum was added to the cell suspension to a 1:4 dilution. After mixing for 5 minutes, the tubes were centrifuged at 500 ***g*** for 1 minute to remove agglutinated red blood cells. Leucocytes in the supernate were then layered onto a solution of 9 per cent Ficoll and 34 per cent Isopaque and centrifuged at 1000 ***g*** for 3 minutes. The white layer at the interface of the plasma and the Ficoll–Isopaque was removed with a Pasteur pipette and washed three times in Hanks's balanced salt solution.

Detection of membrane-bound immunoglobulins

Fluorescein-conjugated antisera (Meloy) to human immunoglobulin determinants were adsorbed with rat liver powder and diluted with phosphate-buffered saline (PBS) to a fluorescein concentration of 30 μg/ml as measured by a Zeiss PMQ II Spectrophotometer as described previously (MacSween and Langley, 1971). Fifty μl fluorescein-conjugated antiserum were added to 1×10^6 lymphocytes suspended in 50 μl of Hanks's balanced salt solution and held at room temperature for 30 minutes. The cells were washed three times in PBS, mixed with a drop of glycerine on a glass microscope slide, and covered with a coverslip. The edges of the coverslip were then sealed with Gestetner correction fluid. Cells with membrane fluorescence were enumerated using a Zeiss ultra-

violet microscope with a vertical illuminator. Specificity was confirmed by showing that the observed fluorescence could be blocked by addition to the fluorescent antisera of immunoglobulins separated from the serum of patients with multiple myeloma (MacSween and Langley, 1971).

Detection of rosette-forming cells

Lymphocytes forming rosettes with sheep erythrocytes were detected by a slight modification of the method described by Brain, Gordon and Willet (1970). Sheep red blood cells were washed in Hanks's balanced salt solution (BSS). Fifty microlitres of BSS containing 8×10^6 sheep red blood cells were mixed with an equal volume of BSS containing 1×10^6 lymphocytes. The cells were centrifuged at 200 ***g*** for 5 minutes and the pellet resuspended on a slowly vibrating mixer for 5 seconds. The number of lymphocytes forming rosettes with at least three sheep red blood cells under these conditions were called active rosettes. The total number of rosette-forming cells was determined by holding the lymphocyte–sheep red cell pellet in ice overnight before resuspending and counting as described above.

Depletion of B cells from whole lymphocyte populations

Lymphocytes were fractionated on IgG–anti-IgG columns (Basten, Sprent and Miller 1972; Wigzell, Sundqvist and Yoshida, 1972; Bankhurst, personal communication). Human IgG was obtained from the sera of patients with myeloma as described by Stanworth (1960). Antisera to human IgG was obtained from New Zealand white rabbits immunized with human IgG in Freund's adjuvant. Antibody activity was confirmed by precipitin reactions with human IgG on Ouchterlony double diffusion plates. Degalan beads (Degussa-Wolfgang, AG) were washed with PBS and held with human IgG in a concentration of 12 mg/ml for 30 minutes at 45° and held overnight at 4°. The beads were washed in PBS, suspended in medium 199, and poured into Pasteur pipettes with the capillary portion plugged with glass wool. The Pasteur pipette columns were filled with a 1:2 dilution of rabbit anti-human IgG in PBS, held at 4° for 2 hours and washed with 7 ml of medium 199. No anti-IgG was detected in the last drop of eluate on double diffusion plates. One to $1{\cdot}5 \times 10^7$ normal lymphocytes in 0·5 ml of medium 199 were applied to the columns, held at 4° for 2 hours, and then eluted with 2·5 ml of medium 199.

A modification of this procedure was introduced to increase the efficiency of separation of leukaemic lymphocytes. Lymphocytes from patients with chronic lymphocytic leukaemia were applied to uncoated Degalan bead columns to remove nonspecifically adherent cells. Cells eluted from these columns were then applied to columns coated with IgG–anti-IgG complexes as described above, eluted, and stained for surface immunoglobulins. The number of lymphocytes applied and the time and temperature at which the cells were held in the uncoated columns were varied to determine optimal recoveries of B cell-depleted lymphocytes. It was found that retention of cells on the uncoated column varied greatly with different cell suspensions. When less than 50 per cent of the lymphocytes applied to the uncoated columns were retained, there was poor depletion of B lymphocytes subsequently on the IgG–anti-IgG column. The procedure which was found to be most satisfactory was to allow 5×10^7 cells to pass directly through an uncoated column without incubation. If more than 50 per cent of the lymphocytes were retained on the uncoated column, the eluted cells were applied directly to IgG–anti-IgG-coated columns. If less than 50 per cent of the cells applied to the uncoated column were retained,

the cells were again applied to an uncoated column, held at room temperature for 15 minutes, and eluted with medium 199. In all cases, this resulted in retention of over 50 per cent of the cells. The eluted cells were then applied to an IgG–anti-IgG column as described above.

Chronic lymphocytic leukaemic lymphocytes with a low density of membrane immunoglobulins were found to separate poorly on IgG–anti-IgG columns. Such cells have been reported to be capable of binding IgG aggregated by heat at 57° (Dickler, Seigal, Bentwick and Kunkel, 1973). Therefore, columns were prepared with Degalan beads coated with heat-aggregated IgG by one of two methods. First, purified IgG (12 mg/ml) was held for 30 minutes at 57° and then added to Degalan beads and held for 30 minutes at 37°. In the second method, a higher concentration of purified IgG (20 mg/ml) was held for 45 minutes at 65°, added to Degalan beads and held for 30 minutes at 37°. The beads were then poured into Pasteur pipette columns and washed with 7 ml of medium 199.

RESULTS

RECOVERY OF LYMPHOCYTES FROM PERIPHERAL BLOOD

An average of 96 per cent of the cells recovered from peripheral blood were mononuclear cells. On light microscopy, 90 per cent of these were lymphocytes and 10 per cent were monocytes. Fifty-three to 62 per cent of the original peripheral blood lymphocytes were recovered.

IDENTIFICATION AND SEPARATION OF NORMAL T AND B CELLS

B cells were identified by the presence of membrane-bound immunoglobulins (Grey *et al.*, 1971), and T cells by the capacity to form rosettes with sheep red blood cells (Brain *et al.*, 1970). Lymphocyte suspensions from fifteen normal individuals were stained for

TABLE 1

PERCENTAGE OF T AND B LYMPHOCYTES IN THE PERIPHERAL BLOOD OF NORMAL INDIVIDUALS BEFORE AND AFTER IgG–ANTI-IgG COLUMN SEPARATION

	Before separation		After separation	
	Mean	Range	Mean	Range
Lymphocytes with membrane-bound IgM (15)*	16·1	9·3–26	1·1	0·5–2·0
Active rosette-forming cells (9)	18·1	10–27	46·1	29–80
Total rosette-forming cells (9)	38·6	20–60	69·1	41–87

* Number of individuals.

membrane-bound IgM before and after separation on IgG–anti-IgG columns. The number of rosette-forming cells in lymphocyte suspensions from nine normal individuals was also determined before and after column separation. The results are shown in Table 1. In two cases, the number of cells with IgG, IgA, kappa and lambda determinants were also assessed. The total proportion of cells with immunoglobulin determinants on this basis was 23 per cent before separation and 2 per cent after column separation.

IDENTIFICATION AND SEPARATION OF T AND B LYMPHOCYTES IN CHRONIC LYMPHOCYTIC LEUKAEMIA

The proportion of IgM-bearing cells in unseparated lymphocyte suspensions from ten patients with chronic lymphocytic leukaemia averaged 68·6 per cent with a range of 44–96. Two per cent of the lymphocytes formed rosettes with sheep erythrocytes. When 5×10^7–13×10^7 lymphocytes were applied to IgG–anti-IgG columns, the proportion of IgM-bearing cells was reduced to an average of 36·4 per cent. It was felt that the poor depletion of B cells was due to the large number of adherent cells. Nonspecifically adherent cells were, therefore, removed on uncoated Degalan bead columns prior to their application to IgG–anti-IgG columns. This resulted in almost complete depletion of B cells in the seven cases with normal density surface immunoglobulin as shown in Table 2. Total rosette forming cells were increased from 2 per cent to 37 per cent by this column separation.

TABLE 2

PERCENTAGE OF T AND B LYMPHOCYTES IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA BEFORE AND AFTER IgG–ANTI-IgG COLUMN SEPARATION

	Before separation		After separation	
	Mean	Range	Mean	Range
Lymphocytes with membrane-IgM of normal density (7)*	67·7	55–80	1·3	0–3
Active rosette-forming cells (2)	2	(2,2)†	31·5	(24,39)†
Total rosette-forming cells (2)	2	(0,4)†	37·0	(28,46)†

* Number of individuals.
† Observed values.

EFFECT OF SURFACE IMMUNOGLOBULIN DENSITY ON SEPARATION OF T AND B CELLS IN CHRONIC LYMPHOCYTIC LEUKAEMIA

Seven patients with chronic lymphocytic leukaemia had B cells with coarse membrane fluorescence similar to that seen in normal individuals. This was interpreted as indicating a normal density of immunoglobulins. The proportions of immunoglobulin-bearing cells and rosette-forming cells on column separation of lymphocytes with this fluorescent pattern is shown in Table 2. It can be seen that this method of separation resulted in satisfactory depletion of B cells with only 1·3 per cent of the recovered cells showing membrane fluorescence. Furthermore, there was recovery of sufficient numbers of viable cells for assessment of their *in vitro* responsiveness. Eighty-five to 90 per cent of the eluted cells were viable on the basis of eosin staining.

Three patients with chronic lymphocytic leukaemia had a finely stippled pattern of membrane fluorescence, indicative of a low density of immunoglobulin. Separation of these lymphocytes resulted in the reduction of fluorescent cells from an average of 68·6–49·9 per cent. Binding of heat-aggregated IgG to chronic lymphocytic leukaemic lymphocytes with low density surface immunoglobulins has been reported (Dickler *et al.*, 1973). Therefore, separation was carried out on heat-aggregated IgG columns, rather than IgG–anti-IgG columns. The results of separation of normal and leukaemic lymphocytes in these

TABLE 3

DEPLETION OF B CELLS ON DEGALAN BEADS COATED WITH HEAT-AGGREGATED IgG

	Percentage of Lymphocytes with membrane-bound IgM			
	Before separation		After separation	
	Mean	Range	Mean	Range
Normal individuals (3)*	18·3	13–22	2·3	2–3
Chronic lymphocytic leukaemia (normal density immunoglobulins) (1)	76		5	
Chronic lymphocytic leukaemia (low density immunoglobulins) (1)	96		95	

* Number of individuals.

columns are shown in Table 3. Lymphocytes effectively depleted of B cells were recovered when cells with normal density surface immunoglobulins were separated on aggregated IgG columns. However, no significant depletion of B cells was observed when lymphocytes with low density surface immunoglobulin from a patient with chronic lymphocytic leukaemia were applied to an aggregated IgG column (12 mg/ml). Therefore, a column was prepared with Degalan beads coated with a higher concentration of IgG (20 mg/ml) at a higher temperature (65°). Lymphocytes from a patient with chronic lymphocytic leukaemia which had lost detectable surface immunoglobulins were passed through this column. The depletion of B cells could not be assessed by surface fluorescence. However, the proportion of T cells forming total rosettes increased from 4 per cent before separation to 40 per cent after separation. This is comparable to the T-cell enrichment obtained when separating leukaemic lymphocytes with normal density surface immunoglobulins (Table 2).

DISCUSSION

Chronic lymphocytic leukaemia, in most instances a disease of B lymphocytes, provides an important model for understanding the function of T lymphocytes in malignancy. Immune responses of T lymphocytes to tumour-related antigens may be important in limiting tumour growth (Sjögren, Hellström, Bansal, Warnar and Hellström, 1972). Chronic lymphocytic leukaemic lymphocytes appear to have surface antigens lacking in normal lymphocytes (Bentwich, Weiss, Sulitzeanu, Kedar, Izak, Cohen and Eyal, 1972). Thus, this disease provides an opportunity to detect and study activity of one lymphocyte population on another with which it is in intimate contact (Fridman and Kourilsky, 1969). A major difficulty in evaluating T-lymphocyte activity against the accumulating B lymphocytes in this disease is in separating the different populations. While in normal subjects, an average of 68 per cent of circulating lymphocytes are T cells, in chronic lymphocytic leukaemia usually less than 16 per cent of peripheral blood lymphocytes are T cells (Jondal, Holm and Wigzell, 1972; Ross *et al.*, 1973; Wybran, Chantler and Fudenberg, 1973). The T cells in this disease are, therefore, diluted with large numbers of B lymphocytes. This change in population may account for the reduced responsiveness of ymphocytes in this disease to stimulation by mitogens such as phytohaemagglutinin

(Bourncle, Klausen and Aschenbrand, 1969; Smith, Cowling and Barker, 1972). To permit evaluation of T-lymphocyte function in chronic lymphocytic leukaemia, it was, therefore, desirable to separate T- and B-cell populations of both normal and leukaemic blood. The function of T cells from patients with chronic lymphocytic leukaemia could then be more accurately compared to the function of T cells isolated from the peripheral blood of normal subjects.

T and B cells in peripheral blood of normal and leukaemic subjects

In both leukaemic and normal subjects, between 53 and 62 per cent of lymphocytes were recovered from the original whole blood lymphocyte population. This separation procedure with Ficoll–Isopaque is a density gradient method and may favour recovery of a less dense lymphocyte population. Surface immunoglobulin was found on 23 per cent of the lymphocytes from normal subjects and 70 per cent of the lymphocytes of patients with chronic lymphocytic leukaemia. Most of this surface immunoglobulin was IgM with only 6 per cent of normal lymphocytes and 2 per cent of leukaemic lymphocytes showing surface IgG or IgA. The same lymphocyte apparently switches over from IgM to IgG synthesis during the normal immune response, and this may explain the presence of immunoglobulins other than IgM on lymphocytes (Levin, Fudenberg, Hopper, Wilson and Nisonoff, 1971). In certain instances, however, binding of cytophilic immunoglobulins in the serum to lymphocytes may account for this phenomenon (Coates and Lennon, 1973).

All ten patients with chronic lymphocytic leukaemia had increased numbers of B cells, indicating accumulation of this cell type. These lymphocytes in individual patients reacted with either anti-kappa or anti-lambda light chains, but not both, in keeping with a monoclonal accumulation of B cells. These findings are similar to those of Preud'homme and Seligmann (1972) and Aisenberg and Block (1972). None of our patients at the time of initial observation lacked an increased population of lymphocytes bearing surface immunoglobulin, although these have been described (Preud'homme and Seligmann, 1972; Dickler *et al.*, 1973).

Two different patterns of membrane fluorescence were observed; one type exhibited by seven patients was patchy and dense; the other, seen in three patients, was finely stippled, suggesting a decreased concentration of surface immunoglobulin. The pattern of fluorescence in any one patient was very consistent from lymphocyte to lymphocyte. Thus, all lymphocytes with surface immunoglobulin in patients with the patchy, dense pattern showed this picture, and all those in the patients with the finely stippled pattern were similarly affected. Patients with the finely stippled pattern of lymphocyte surface immunoglobulin had percentages of B cells similar to patients with the dense pattern. Such cells may correspond to the heavily and lightly labelled lymphocytes as determined by autoradiography by Wilson and Hurdle (1973).

Some of our patients have been followed over a period of time, but only one patient to date has shown a change in the fluorescence pattern of surface immunoglobulin. This patient initially had a finely stippled pattern of fluorescence. Three months later, no surface immunoglobulin could be detected on several determinations. There was no change in his clinical disease which is asymptomatic and which has not required treatment for the 10 years he has been under observation. McLaughlin, Wetherly-Mein, Pitcher and Hobbs (1973) studied ten patients with chronic lymphocytic leukaemia within 3 years of diagnosis. All had a patchy, dense surface fluorescence. Three other patients first studied 8 years after diagnosis had a finely stippled pattern. They suggested that the dura-

tion of the disease was one factor that resulted in varying amounts of surface immunoglobulins. That there are other factors is suggested by our studies. Thus, three of our four patients with chronic lymphocytic leukaemia of longest duration, that is 9–10 years from diagnosis, had a dense pattern of surface fluorescence, while two of our five with the shortest known duration of disease had a finely stippled pattern. However, those patients with the shortest known duration of disease and a finely stippled pattern also had more aggressive disease. Further, chronic lymphocytic leukaemia is a disease capable of existing for long periods without symptoms, so that it is difficult to be sure that recent presentation means recent onset of disease.

The ability of lymphocytes to adhere to sheep red blood cells to form rosettes is considered to be an indication that such lymphocytes are T cells (Brain *et al.*, 1970; Wybran *et al.*, 1973). The number of rosettes is increased if the cells are held at low temperatures. In the normal subjects studied, 38 per cent of lymphocytes formed rosettes (18 per cent immediately and an additional 18 per cent with incubation at 0°). Only 0–4 per cent of the lymphocytes from patients with chronic lymphocytic leukaemia formed rosettes, providing further support for the view that these leukaemic lymphocytes are B cells.

Separation of T cells from whole lymphocyte populations

When lymphocytes containing an average of 16·1 per cent B cells obtained from the peripheral blood of normal subjects were applied to IgG–anti-IgG columns, the eluted cells contained an average of 1·1 per cent B cells. While B cells are more adherent to surfaces than T cells (Adams, 1973), when normal lymphocytes containing 17 per cent B cells were passed through uncoated Degalan bead columns, 11 per cent of the eluted lymphocytes were B cells. Therefore, the adherence of B cells on the IgG–anti-IgG columns was largely specific binding to IgG–anti-IgG complexes. It has been postulated that B cells contain a receptor for the Fc portion of antibody molecules bound to antigen (Basten *et al.*, 1972). Therefore, depletion of B cells on the IgG–anti-IgG columns might be due to binding of B cells to the Fc portion of the rabbit anti-IgG. However, anti-light chain antibodies were not adsorbed from the rabbit anti-IgG so that IgM-bearing lymphocytes containing light chains could also be bound to excess anti-light chain determinants on the column. Total rosettes increased from 38·6 to 69·1 per cent, confirming that the lymphocytes eluted from the IgG–anti-IgG columns were significantly enriched for T cells.

When lymphocytes from patients with chronic lymphocytic leukaemia were applied directly to IgG–anti-IgG columns, proportionately few B lymphocytes were removed. This failure to remove B cells in this condition might have been due to overloading the column with large numbers of adherent cells. Chronic lymphocytic leukaemic lymphocytes were, therefore, applied to a preliminary column of uncoated Degalan beads to remove nonspecifically adherent cells. When approximately 50 per cent of the chronic lymphocytic leukaemic lymphocytes were retained on the preliminary column, satisfactory depletion of B cells occurred with application of this column eluate to an IgG–anti-IgG column. When more than 70 per cent of the lymphocytes were retained on the preliminary column, insufficient lymphocytes were obtained for subsequent studies. The number of lymphocytes retained on the preliminary uncoated column varied greatly from one cell suspension to another and was influenced by the number of lymphocytes applied to the column, the time they were held on the column, and the temperature at which the experiments were done. Recovery of cells from the column was decreased if more than 5×10^7 cells were applied. Decreasing recoveries were also observed, the longer cells were held on the

columns. Recoveries were several fold greater when the experiments were carried out at 4° compared to room temperature.

A procedure was developed which enabled a predictable proportion of cells to be retained on the uncoated column. The suspension with markedly adherent cells could be utilized immediately after passage without incubation through an uncoated column. Suspensions with fewer adhering cells required an additional 15 minutes incubation at room temperature in the uncoated column before elution. In either case, subsequent application of these suspensions, depleted of adherent cells, to IgG–anti-IgG columns resulted in satisfactory depletion of B cells.

After column passage, rosette-forming lymphocytes from patients with chronic lymphocytic leukaemia increased from 2 to 37 per cent. However, the sum of rosette-forming and immunoglobulin-bearing lymphocytes indicated a large proportion of cells had neither characteristic, in contrast to normal lymphocytes. This might represent an increase in cells with neither the characteristics of T or B cells, or these eluted cells might have a lower density of T-cell receptors for sheep red blood cells, analogous to the lower density of immunoglobulins on B cells in chronic lymphocytic leukaemia. Whether decreased proportions of rosette-forming cells in the purified T-cell population in chronic lymphocytic leukaemia represents a decreased reactivity of T cells is currently under investigation by further studies on T-cell responses to stimulation by mitogens and antigens.

Chronic lymphocytic leukaemic lymphocytes with low density surface immunoglobulins

It was consistently found that chronic lymphocytic leukaemic lymphocytes with low density surface immunoglobulins were poorly depleted of B cells on column separation. This suggested that an important mechanism of B-cell adherence was related to binding to the anti-immunoglobulins coating the column beads. It had previously been reported that chronic lymphocytic leukaemic lymphocytes with low density of immunoglobulins will bind heat-aggregated IgG (Dickler *et al.*, 1973). An attempt was therefore made to remove these low density immunoglobulin-bearing lymphocytes by using a column of Degalan beads coated with IgG aggregated at 57°. Lymphocytes with a normal density of immunoglobulin were removed by these columns, but preliminary attempts failed to obtain a satisfactory depletion of those B lymphocytes with a low density of surface immunoglobulins. However, a column was prepared with a higher concentration of IgG aggregated at 65°. When the lymphocytes from the patient whose cells had lost all detectable immunoglobulins were passed through this column, the number of rosette-forming lymphocytes increased from 4 to 40 per cent. This was comparable to the T-cell enrichment seen when lymphocytes bearing normal density surface immunoglobulin from patients with chronic lymphocytic leukaemia were passed through IgG–anti-IgG columns. This suggested that lymphocytes with low density surface immunoglobulins might bind to Degalan beads coated with higher concentrations of aggregated IgG.

Different techniques used to obtain enriched populations of T cells may not yield comparable cell populations. Wybran *et al.* (1973) obtained T lymphocytes from patients with chronic lymphocytic leukaemia on the basis of their ability to form rosettes with sheep red blood cells and to subsequently sediment. The T-cell population recovered in our experiments were selected because they were not adherent to the Degalan beads and antigen–antibody complexes. There are reports that early reactive T cells in mice are more adherent than non-reactive T cells (Shortman, Byrd, Williams, Brunner and Cerottini, 1972). It is, therefore, possible that the eluted T-cell population in our experi-

ments contain fewer 'activated' T cells. Our initial separation of lymphocytes from peripheral blood favoured the recovery of low density lymphocytes which are said to be more reactive than high density cells (Shortman *et al.*, 1972). The two separation procedures, therefore, tend to offset each other in these selection factors. In any event, the separated lymphocytes from patients with chronic lymphocytic leukaemia were largely depleted of B cells and enriched for T cells. These lymphocytes were found to be viable and to respond to mitogen stimulation *in vitro* (Fernandez, MacSween and Langley, in preparation).

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