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# Hypogammaglobulinaemia associated with abnormalities of both B and T lymphocytes in patients with chronic lymphatic leukaemia

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SUMMARY

The underlying basis for hypogammaglobulinaemia in patients with chronic lymphatic leukaemia (CLL) was investigated by measurement of immunoglobulin produced *in vitro* in cultures of pokeweed mitogen-stimulated B and T lymphocytes. B and T cells were separated by sheep red blood cell rosette techniques and, by culture of these cells from CLL patients in various combinations with B or T cells from normal subjects, it was possible to measure independently the function of B lymphocytes and the helper or suppressor function of T lymphocytes.

By these methods it was found that the B lymphocytes of six of eight patients failed to produce immunoglobulins *in vitro*. B lymphocytes from two patients appeared to produce immunoglobulins *in vitro*. T lymphocytes from five of the eight patients had low or undetectable helper T cell function and in six patients their T lymphocytes had excessive suppressor activity in comparison to T lymphocyte populations from normal subjects. Whether the primary abnormality in the CLL T cell populations was a deficiency of helper T cells or excess of suppressor T cells was uncertain from these studies. These results suggest that immunoglobulin production by B lymphocytes from most patients with CLL was abnormal but also that T cells from CLL patients may be abnormal in respect to their role in immunoglobulin production at an early stage of the disease. These findings may assist in understanding the pathogenesis of this disease and lead to new approaches in treatment.

## INTRODUCTION

Infections related to hypogammaglobulinaemia may be an early feature in chronic lymphatic leukaemia (CLL) and assume more clinical importance than the haematologic manifestations of this disease (Miller & Karnofsky, 1961; Cone & Uhr, 1964). There is general agreement that the low levels of immunoglobulins are due to deficiency in production by B lymphocytes (Wells & Fudenberg, 1971) but the factors responsible for this are unknown.

New insights into the hypogammaglobulinaemia in certain clinical disorders have been provided by recent studies showing that immunoglobulin production is regulated by helper T cells and suppressor cells of the T cell or macrophage lineage (Broder, Muul & Waldmann, 1978). In particular, in patients with acquired idiopathic hypogammaglobulinaemia and multiple myeloma it was suggested that hypogammaglobulinaemia may be caused by overactivity of suppressor cells against immunoglobulin production (Waldmann *et al.*, 1974; Broder *et al.*, 1975).

The purpose of the present study was to investigate whether similar defects in regulator cells may

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underlie the hypogammaglobulinaemia in patients with CLL or whether this is due solely to abnormalities in B lymphocytes. Our results suggest that hypogammaglobulinaemia in this disorder may be due to a spectrum of abnormalities including those of B lymphocytes and helper T cells. Overactivity of suppressor T cells was a common finding but may be secondary to abnormal helper cell function in most of the patients.

## MATERIALS AND METHODS

Abnormalities in the production of immunoglobulins by CLL patients were investigated by measurement of immunoglobulins produced in cultures of pokeweed mitogen-stimulated (PWM) B and T lymphocytes. This involved culture of separated B lymphocytes with varying proportions of T lymphocytes and measurement of immunoglobulins released into the culture fluid as outlined below.

*Assay of immunoglobulins.* Immunoglobulins produced in the *in vitro* cultures were estimated by nephelometric methods using a Hyland PDQ laser nephelometer by a method similar to that described by Deaton *et al.* (1976). Eight standards over the range from 60 to 8000 ng/ml were prepared by dilution of the standards supplied by Hyland for routine estimation of IgG, IgM and IgA. Antisera to IgM, IgA and IgG were from Dako (Copenhagen) The IgM (lot 088A) and IgA (lot 018F) antisera were used in a final dilution of 1 :1000 in nephelometer buffer. The IgG antiserum (lot 048B) was absorbed on foetal calf serum bound to sepharose 4B by cyanogen bromide and used at a dilution of 1 :100. Supernatants from four *in vitro* cultures were pooled into 2 × 1 ml samples. They were centrifuged at 2000 *g* for 30 min and duplicate 100 $\mu$l samples were used for assay of IgM, IgA and IgG and sample blanks. One hundred microlitres of RPMI 1640 (GIBCO)+10% foetal bovine serum (FBS) were added to the standards to make the assay fluid similar to that of the samples from tissue cultures.

*Procedure.* One hundred microlitres of the standards and test samples were added to 920 $\mu$l of the appropriate antibody solutions and incubated for 1–3 hr at room temperature. Sample blanks and standard blanks were prepared by the addition of 920 $\mu$l of filtered nephelometric buffer alone. Settings were adjusted to zero with saline in buffer blank. Compensation for relative light scatter (RLS) due to the Dako antibody was provided by a second adjustment using a 1 :1000 dilution of the antibody in saline in the antibody blank. Sample blanks consisted of 100 $\mu$l of supernatant and 920 $\mu$l of nephelometer buffer. The sensitivity of the machine was adjusted to give a maximum RLS reading for the highest 8000 ng standard.

Settings were adjusted to zero with the buffer and all tubes then read by the procedures described by the manufacturer.

*E-rosettes.* Estimation of rosette formation was carried out by the method of Kaplan & Clark (1974) using aminoethylisothiouronium bromide (AET) treated sheep red blood cells (SRBCs). Equal proportions of 1% AET-treated SRBCs and mononuclear cells at $2 \times 10^6$/ml in RPMI+10% FBS were mixed and incubated at 37°C for 15 min. The mixture was centrifuged at 300 *g* for 5 min and incubated at 4°C for 1 hr. The pellet was resuspended by gentle shaking and the percentage of E-rosettes estimated using a counting chamber and phase microscopy.

*B lymphocytes.* Cells with surface immunoglobulins were detected by the use of fluorescein-labelled polyvalent sheep anti-human immunoglobulin (Wellcome reagents). In order to remove non-specifically bound surface immunoglobulins, $5 \times 10^5$ lymphocytes were exposed to acetate buffer at pH 4·5 for 1 min at 4°C (Kumagai *et al.*, 1975) and then washed in phosphate buffered saline (PBS). The cells in 50 $\mu$l PBS were incubated in 100 $\mu$l of a 1 : 8 dilution of the antiserum for 20 min at room temperature and washed three times in PBS. They were then mounted in PBS : glycerol pH 8·2 and examined by fluorescent microscopy.

*Cell separation procedures.* Mononuclear cells were separated from defibrinated blood samples of patients with CLL and age and sex matched normal controls by centrifugation on a Ficoll–Hypaque mixture as described by Böyum (1968). Cells forming E-rosettes (E+ cells) were separated from non-E rosette (E−) forming cells by centrifugation on the Ficoll–Hypaque mixture as described previously (Hersey, Edwards & Edwards, 1976). The E-rosette-forming (E+) cells passed through the Ficoll–Hypaque to the bottom of the tube and were separated from the SRBCs by lysis of the SRBCs with a hypotonic solution (formed by mixing 1 volume of Hanks' buffered salt solution (HBSS) with 2 volumes of distilled water) for 30 sec. The E-rosette and separation procedure was repeated on the E+ and E− populations to obtain relatively pure populations of E+ and E− cells.

*In vitro assay procedure.* This was adapted from that described by Moretta *et al.* (1977). Thus, $10^5$ E− (B) lymphocytes from the CLL patients and normal controls were cultured alone or with $10^4$, $10^5$ and $3 \times 10^5$ E+ cells from either the CLL patients or the normal control. In addition, cultures of E− and E+ cells from the normal control at optimal concentrations for immunoglobulin production ($10^5$ E− + $3 \times 10^4$ E+ cells) were cultured with $10^4$, $3 \times 10^4$, $10^5$ or $3 \times 10^5$ E+ cells from the CLL patient to test for suppressor activity in E+ population of the patient.

Pokeweed mitogen (PWM) (GIBCO batch A771101) was used to stimulate antibody production at a final dilution of 1 : 200 which was the dilution shown in prior experiments to produce optimal immunoglobulin production *in vitro*. Cultures were in groups of six 12 × 75 mm round-bottomed polystyrene tubes (RT30 Filtrona, Melbourne) and tissue culture fluid was RPMI 1640 (GIBCO) + 10% FBS (Australian Laboratory Service batch 64). Final volume in each tube was 0·5 ml. Control cultures of E+ and E− cells were included without PWM. The tubes were capped and incubated at 37°C in 5% $CO_2$, 95% air atmosphere for 7 days without change of the medium. At the end of this period the supernatants from four tubes were collected for assay of immunoglobulin production. Cell division was assessed in the remaining two cultures by the

addition of 2 $\mu$Ci $^{125}$I-iododeoxyuridine ($^{125}$I UDR) for 4 hr. $^{125}$I UDR incorporation was determined by washing the cells twice in saline and twice in 5% trichloroacetic acid. The tubes were then counted in a gamma counter and results were expressed as counts per minute corrected for $^{125}$I decay.

*Patients.* The relevant clinical details of the eight patients studied, including blood leucocyte counts, cell marker studies, immunoglobulin levels and treatment, are shown in Table 1. Two patients had received no treatment, one had been treated by leucapheresis only and the other five had received chlorambucil and/or prednisolone for varying periods. All patients had abnormally low immunoglobulins of one or more classes.

TABLE 1. Clinical and laboratory data on patients with CLL

| Subject | Sex | Age | Date of diagnosis | Leucocyte count ($10^6$/ml) | Immunoglobulin levels (mg%) IgG | IgM | IgA* | E rosette (%) | Ig +ve cells (%) | Treatment |
|---|---|---|---|---|---|---|---|---|---|---|
| C.A. | M | 81 | 1969 | 52 | 32 | 17 | 70 | 3 | 34 | C 2 mg, 1973<br>C 2 mg P 7–10 mg, 1975 |
| S.C. | M | 63 | 1978 | 116 | 629 | 36 | 47 | 1 | 38 | C 2 mg alt. days |
| D.R. | M | 78 | 1978 | 108 | 246 | 179 | 36 | 3 | 62 | C 5 mg, P 5 mg/day |
| B.W. | F | 48 | 1977 | 19 | 1120 | 113 | 47 | 6 | 75 | Nil |
| M.W. | F | 84 | 1972 | 52 | 584 | 27 | 119 | 16 | 69 | C 5 mg, P 5 mg/day |
| N.M. | F | 72 | 1976 | 94 | 350 | 37 | 47 | 5 | 62 | Nil |
| P.S. | M | 48 | 1976 | 296 | 700 | 42 | 46 | < 1 | 43 | Nil for 6 months. Previously C & P |
| P.G. | M | 57 | 1976 | 98 | 510 | 2 | 54 | 10 | 57 | Leucapheresis |

C = chlorambucil, P = prednisolone.
* Normal immunoglobulin levels: IgG > 800 mg%, IgM > 70 mg% and IgA > 90 mg%.

## RESULTS

### *Control studies*

*Detection of low concentrations of immunoglobulin by laser nephelometry.* Standard curves obtained in the nephelometer which were typical of those obtained throughout these studies are illustrated in Fig. 1. A linear relationship between the percentage of relative light scatter (% RLS) and the concentration of the immunoglobulins was noted over a concentration range of 40–8000 ng/ml. The straight line correlations for the assays shown were 0·996 for IgG, 0·9996 for IgM and 0·9996 for IgA.

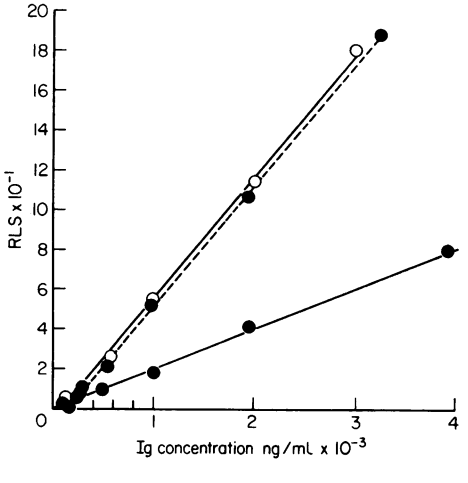


FIG. 1. Linear relationship between relative light scatter (RLS) and immunoglobulin levels over the range 40–4000 ng/ml is shown using the Hyland PDQ nephelometer. (●—●) IgG, (○—○) IgM, (● - - - ●) IgA.

The reproducibility of the assays at low concentrations of immunoglobulins was determined by repeated assays of ten samples with the same concentrations of immunoglobulins containing approximately 100 ng/ml. The mean$\pm$1 standard deviation for IgG, IgM and IgA were 30·1$\pm$2·3% RLS, 59·3$\pm$2·8% RLS and 56·1$\pm$3·1% RLS respectively.

*Specificity of the antisera.* The antisera to the different classes of immunoglobulin were tested against purified immunoglobulins of each class. The results in Table 2 indicate that there was no significant cross reactivity of the antisera with different immunoglobulin classes.

*Degree of separation of E+ and E− cell populations.* Table 3 indicates the mean values of the marker studies on the E+ and E− populations of eleven normal subjects and of five patients obtained during the period of these studies. Similar degrees of separation were obtained on the leucocyte populations from normal and leukaemic blood.

*Production of immunoglobulins in vitro by B cells from normal subjects.* The mean immunoglobulin levels in supernatants of $10^5$ B lymphocytes stimulated with PWM and varying numbers of syngeneic or allogeneic T lymphocytes from different normal subjects are shown in Fig. 2. Fig. 2 also illustrates the values when B lymphocytes from normal subjects were cultured with $0{\cdot}3 \times 10^5$ syngeneic T lymphocytes together with varying numbers of allogeneic T lymphocytes. The results indicate that in cultures

TABLE 2. Specificity of antisera for IgG, IgM or IgA

| Antiserum | Ig added ($\mu$l) | Per cent RLS |
|---|---|---|
| Anti-IgG | 100 G | 165·5 |
| | 100 G + 100 M | 164·0 |
| | 100 M | 3·0 |
| | 100 G + 100 A | 158·5 |
| | 100 A | 3·2 |
| Anti-IgM | 100 M | 171·4 |
| | 100 M + 100 G | 171·7 |
| | 100 G | 3·7 |
| | 100 M + 100 A | 173·1 |
| | 100 A | 5·7 |
| Anti-IgA | 100 A | 154·9 |
| | 100 A + 100 G | 152·7 |
| | 100 G | 2·2 |
| | 100 A + 100 M | 156·4 |
| | 100 M | 3·6 |

Concentrations: IgG 140 $\mu$g/ml, IgM 130 $\mu$g/ml, IgA 130 $\mu$g/ml.

TABLE 3. Cell surface marker studies on cell populations added to cultures

| Subjects | B population | | T population | |
|---|---|---|---|---|
| | Ig+ve (%) | E+cells (%) | Ig+ve (%) | E+cells (%) |
| Normals (11) | 85·9$\pm$5·5 | 1·9$\pm$2·0 | 2·4$\pm$1·8 | 86·4$\pm$4·8 |
| Patients c CLL | | | | |
| M.W. | 94 | 3·9 | 0 | 62 |
| N.M. | 78 | 6 | 1 | 98 |
| P.G. | 86 | 1 | 3 | 81 |
| D.R. | 92 | 0 | 6 | 85 |
| B.W. | 87 | 4 | 6 | 79 |

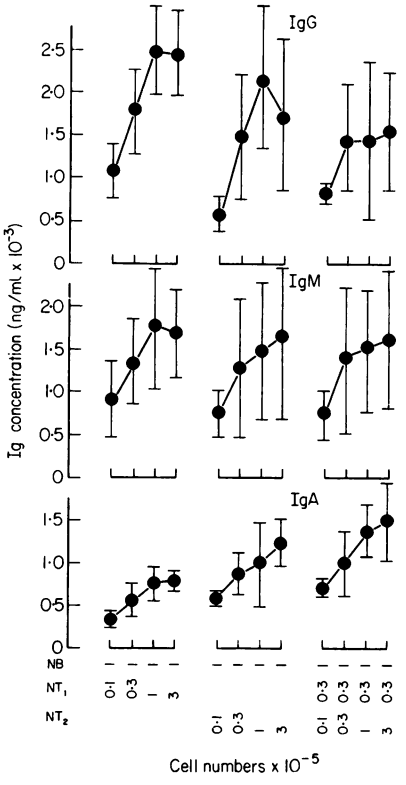


FIG. 2. Production of immunoglobulins *in vitro* by B cells from normal subjects. The numbers of B and T cells in each culture are indicated along the abscissa. The values for studies on syngeneic B and T cells (NB, NT) are means ± 1 s.d. of eleven studies and those for studies involving allogeneic T cells ($NT_2$) means ± 1 s.d. of four studies.

with syngeneic B and T lymphocytes plus varying numbers of allogeneic T lymphocytes, IgG production was slightly less than in cultures with equivalent numbers of T lymphocytes from a single donor. There was, however, a similar dose–response relationship with increasing numbers of T lymphocytes.

*Immunoglobulin production in cultures of B and T lymphocytes from patients with CLL*

The various combinations of E+ (T) and E− (B) cells in the cultures allowed separate analysis of B cell, helper T cell and suppressor T cell function. The extent to which defects in the function of these different cell types contributed to the abnormal immunoglobulin production *in vitro* varied between patients. The results below are grouped, therefore, according to the main abnormalities detected in the *in vitro* cultures.

*Low immunoglobulin levels associated with abnormal B cell function, low helper cell function and increased suppressor cell activity.* Abnormal B cell function was shown in these studies by low production of immunoglobulins in cultures in which B cells from CLL patients were cultured together with increasing numbers of T cells from the patient or from normal subjects. An example of such a study is that of M.W. in Fig. 3. As shown in Table 1 this 84-year-old woman had a leucocyte count of $52 \times 10^6$/ml and low serum IgG (584 mg/100 ml) and IgM (27 mg/100 ml) levels. She had been treated intermittently with chlorambucil and prednisolone for 2 months preceding these studies. B lymphocytes from the patient did not produce immunoglobulin in the presence of her own T lymphocytes (Fig. 3a) or of allogeneic T lymphocytes from a normal control (Fig. 3d). This is interpreted as indicating abnormal B cell function.

In addition, B lymphocytes from the normal control did not produce immunoglobulin in the presence of T lymphocytes from the patient (Fig. 3e), whereas they did so when cultured with the control's own T

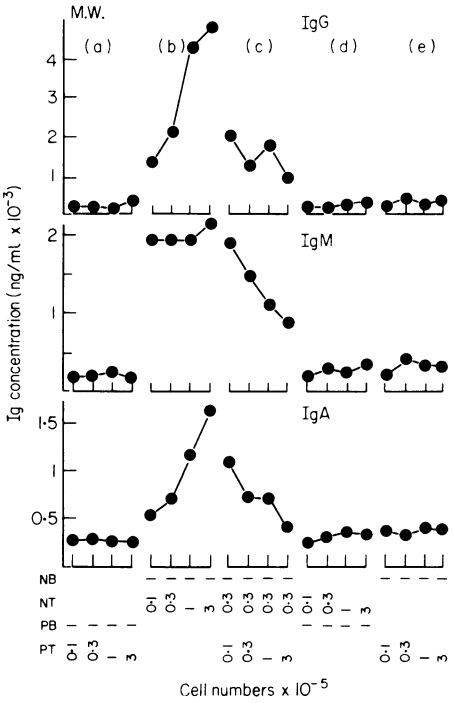


FIG. 3. Abnormalities in B cell function are shown by low immunoglobulin production in cultures of B cells from CLL patients with autologous T cells (a) or allogeneic normal T cells (d). This was associated with abnormalities of helper T cell function shown in (e) and excessive suppressor T cell function (c). Production of immunoglobulins by B cells from the normal donor in the presence of autologous T cells is indicated by cultures under (b). Numbers at the bottom of the Figure indicate numbers of cells added to each culture. NB and NT = normal B cells and normal T cells respectively; PB and PT = CLL patient B cells and T cells respectively. Standard errors not shown but these were less than 10% for all cultures.

lymphocytes (Fig. 3b). This indicated normal helper T cell function in control lymphocytes but absence of T cell function in those from the patient. The patient's T lymphocytes still retained suppressor activity as shown by their marked suppression of immunoglobulin synthesis when they were cultured with normal B and normal T lymphocytes, as shown in Fig. 3c.

This pattern of abnormalities, i.e. abnormal B cell and helper T cell function with evidence of suppressor T cell function was shown by four patients in the study (M.W., C.A., S.C. and P.G.).

M.W. and C.A. had advanced CLL of several years' duration and had received therapy with prednisolone and chlorambucil. S.C. and P.G. had recently diagnosed CLL. P.G. was untreated and S.C. had received 2 mg chlorambucil on 2 days preceding the study.

*Normal production of immunoglobulin by B lymphocytes from CLL patients.* Fig. 4 illustrates a study on B.W., a 48-year-old woman with early untreated CLL (leucocyte count 19 × $10^9$/l) and low IgA levels (47 mg/100 ml). The B lymphocytes from the patient appeared to produce immunoglobulins of all three classes (Fig. 4a and d) comparable with those from a normal control. Helper T cell function (Fig. 4c) also appeared normal. When T lymphocytes from this patient were added to the cultures of control B and T cells (Fig. 4c), however, marked suppression of IgM, IgA and to a lesser extent IgG production was observed. Evidence of suppression at higher numbers of T cells was also evident in cultures (Fig. 4a) and (Fig. 4e) in that immunoglobulin production did not increase beyond a ratio of 1:0·3 for B and T cells whereas the optimal ratio for the normal control was 1:1 or 1:3 B to T cells.

The second patient with B lymphocytes showing immunoglobulin production *in vitro* was N.M., a 72-year-old woman with established CLL. No treatment with chlorambucil or prednisolone had been given to the patient for 12 months before study. Significant production of IgG and small amounts of IgA and IgM was detected in the presence of autologous and allogeneic T cells. Allogeneic helper T cell function

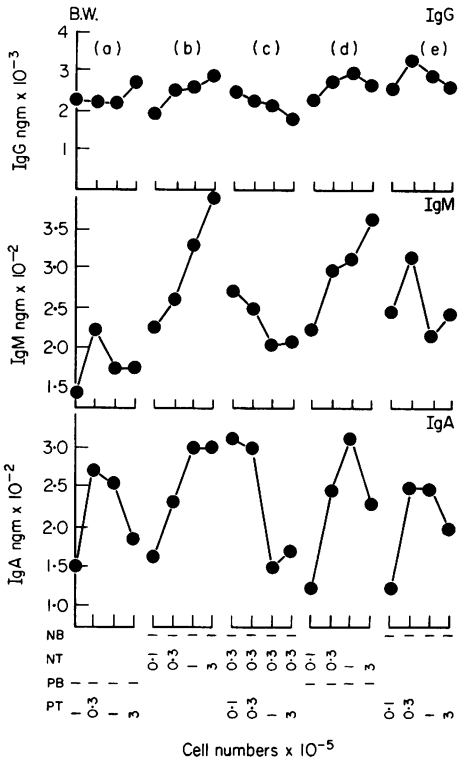


FIG. 4. Example of excessive suppressor T cell activity in patient with CLL. *In vitro* production of immunoglobulins was shown in cultures of CLL B lymphocytes (a,d). T cells from this patient had marked suppressor activity when added to cultures of normal B and T cells as shown under (c). This was particularly pronounced for IgM and IgA production. Helper function was evident in T cells from this patient shown under (e). See also legend to Fig. 3.

was absent in the cells of this patient and suppressor cell activity was not pronounced. Studies on this patient are to be reported elsewhere.

*Low immunoglobulin levels associated with abnormal B cell function, and normal helper cells.* Fig. 5 illustrates a study on patient P.S., aged 48, with untreated CLL and low serum IgG (700 mg/100 ml), IgM (42 mg/100 ml) and IgA (46 mg/100 ml) levels. His B lymphocytes did not produce immunoglobulin in the presence of his own or the control T lymphocytes (Fig. 5a) and (Fig. 5d). His T lymphocytes induced immunoglobulin production in normal B lymphocytes (Fig. 5c) indicating normal helper T cell function. Suppressor activity was evident, however, in these cultures at ratios greater than 1 : 1, B to T cells. Normal Ig synthesis was suppressed markedly in cultures where the patient's T lymphocytes were added to cultures of normal B and T cells (Fig. 5c). The same pattern was evident for all immunoglobulin classes in this patient.

A further variation in the results of these studies was found in the study on D.R. (unreported). This patient had advanced CLL with low IgG (246 mg/100 ml) and IgA (36 mg/100 ml) levels and had been treated with chlorambucil and prednisolone. His B lymphocytes showed low immunoglobulin production in the presence of both his own T cells and the control T cells. In cultures designed to test for suppressor cell activity, helper cell activity was evident for IgM and IgA production but no suppressor cell activity was detected.

## DISCUSSION

*In vitro* culture of blood lymphocyte populations has been used to investigate abnormalities of immunoglobulin production in such conditions as idiopathic acquired hypogammaglobulinaemia (Waldmann *et*

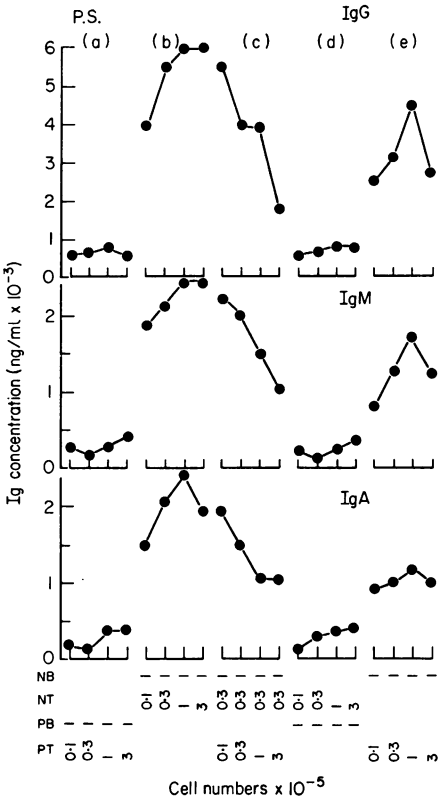


FIG. 5. Example of abnormal B cell function and excessive suppressor T cell function in patient with CLL. Low immunoglobulin production was seen in cultures with CLL B lymphocytes (a) and (d). Normal helper T cell function to allogeneic normal B cells was shown in cultures under (e). Excessive suppressor T cell activity against immunoglobulin production was shown for all immunoglobulin classes (c). See also legend to Fig. 3.

*al.*, 1974), multiple myeloma (Broder *et al.*, 1975) and acute leukaemia (Broder *et al.*, 1978). In addition, *in vitro* cultures of B and T cell enriched populations were used to analyse helper effects of T cells (Keightly, Cooper & Lawton, 1976) and the nature of T cells suppressing immunoglobulin production (Moretta *et al.*, 1977). In the present study this approach was developed further to allow separate analysis of B cell, helper T cell and suppressor T cell function in patients with CLL.

*Lack of B cell function*

The commonest and perhaps not unexpected finding was that production of immunoglobulin by the B enriched populations in the presence of the patients' own T cells or of normal T cells was abnormally low in all but two of the patients studied. One of the latter patients had early untreated CLL and the immunoglobulin production may have resulted from surviving normal B lymphocytes. The second patient had advanced CLL and immunoglobulin production was confined largely to the IgG class. This may be an example of *in vitro* differentiation and immunoglobulin synthesis by CLL lymphocytes induced by PWM as previously reported in two cases of CLL by Fu *et al.* (1978). Further details of this study will be reported elsewhere.

The validity of testing for B cell function by co-culture with allogeneic T cells from normal subjects could be questioned. The culture conditions would appear favourable for generation of cytotoxic T cells against the B cells and so lead to reduction of immunoglobulin synthesis. This did not appear to occur in previously reported studies (Keightly *et al.*, 1976; Broder *et al.*, 1978) or in the present study. When B cells from normal subjects were co-cultured with allogeneic T cells from normal subjects, immunoglobulin production was dependent on the number of T cells in culture. Failure of immunoglobulin production in

the presence of PWM and normal T cells therefore appeared a reliable index of abnormalities in the B cells.

*Abnormalities in helper and suppressor T cell function*

The control studies also suggested that the *in vitro* culture system could be used as a reliable indicator of helper and suppressor T cell function. Failure of B lymphocytes from normal subjects to produce immunoglobulin in the presence of increasing numbers of allogeneic T lymphocytes was not seen in the studies on normal subjects and hence this finding with T cells from CLL patients suggested abnormal helper T cells in these patients. By these criteria T cells from five of eight patients with CLL had low or absent helper activity.

Addition of T lymphocytes from CLL patients to mixtures of normal B and T cells produced suppression of immunoglobulin synthesis at T:B cell ratios of less than 1:1 in six cases. In control experiments, addition of allogeneic T cells from normal subjects did not cause suppression until the ratio of T:B cells exceeded 3:1. T cell populations of normal subjects are belived to contain both helper and suppressor cell activity for immunoglobulin synthesis (Moretta *et al.*, 1977). Our findings of more pronounced suppressor activity in T cell populations from CLL patients, relative to those from normal subjects, could be interpreted either as indicating an excess of suppressor T cells or a relative decrease in helper cells. This question is currently being examined by study of helper activity in T cell populations depleted of suppressor T cells bearing Fc receptors for IgG (T$\gamma$+ cells) as described by Moretta *et al.* (1977). Initial results from these studies suggest an absolute deficiency of helper T cells rather than a primary excess of suppressor T cells.

T cell populations from CLL patients in several instances exhibited suppression of immunoglobulin synthesis *in vitro* even though the *in vivo* levels of immunoglobulins in the patient were within normal limits. This finding again may indicate that the suppressor activity detected in these assays is not the primary abnormality but secondary to defects in helper cell activity. In the instances referred to above, helper activity may still have been sufficient to support normal immunoglobulin synthesis *in vivo*. Alternatively, suppressor and helper cell activity in the blood may not be representative of that in other areas of the lymphoid system such as the lymph nodes.

Our reason for initiating these studies was the belief that B cell abnormalities alone were unlikely to explain the marked hypogammaglobulinaemia, often affecting only one class of antibody, seen in patients with CLL. These abnormalities are seen commonly at an early stage of the disease when the number of normal B lymphocytes present might not be expected to be significantly reduced. The present results support the suggestion that abnormalities in T cells may be implicated in the early defects of immunoglobulin synthesis in these patients.

The possibility that T cells may be abnormal in patiets with CLL has been much discussed. Abnormalities in the response of lymphocytes from CLL patients to the T cell mitogen phytohaemagglutinin (PHA) have been found repeatedly (Shaw *et al.*, 1974). This might be due to dilution of normal T cells by the neoplastic B lymphocytes (Wybran, Chantler & Fudenberg, 1973), or to intrinsic defects in the response of CLL T cells to PHA (Fernandez, MacSween & Langley, 1977). Abnormalities in helper T cell function in two patients with CLL were reported by Fu *et al.* (1978), but Fauci, Pratt & Whelan (1977) found no abnormalities in this function.

Abnormalities in T cells in CLL patients may indicate that the origin of the neoplastic process in CLL may be in a precursor stem cell for both T and B cell development analogous to that described in Philadelphia chromosome positive chronic myeloid leukaemia (Greaves & Janossy, 1978).

Further studies to delineate more precisely the T cell abnormalities in CLL and their clinical significance are in progress. Appreciation of CLL as a disease of both T and B cells, however, may explain the frequent occurrence of viral infections and second neoplasms (Gunz & Angus, 1965) in these patients and may lead to new therapeutic approaches to treatment of the disease and its complications.

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## REFERENCES


BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. Lab. Invest.* **21**, 77.

BRODER, S., HUMPHREY, R., DURM, M., BLACKMAN, M., MEADE, D., GOLDMAN, C., STROBER, W. & WALDMANN, T. (1975) Impaired synthesis of polyclonal (non-paraprotein) immunoglobulins by circulating lymphocytes from patients with multiple myeloma. *N. Engl. J. Med.* **293**, 887.

BRODER, S., MUUL, L. & WALDMANN, T.A. (1978) Suppressor cells in neoplastic disease. *J. natl. Cancer Inst.* **61**, 5.

BRODER, S., POPLACK, D., WHANG-PENG, J., DURM, M., GOLDMAN, C., MUUL, L. & WALDMANN, T.A. (1978) Characterization of a suppressor-cell leukemia. Evidence for the requirement of an interaction of two T cells in the development of human suppressor effector cells. *N. Engl. J. Med.* **298**, 66.

CONE, L. & UHR, J.W. (1964) Immunological deficiency disorders associated with chronic lymphatic leukemia and multiple myeloma. *J. clin. Invest.* **43**, 2241.

DEATON, C.D., MAXWELL, K.W., SMITH, R.S. & CREVELING, R.L. (1976) Use of laser nephelometry in the measurement of serum proteins *Clin. Chem.* **22**, 1465.

FAUCI, A.S., PRATT, K.R. & WHELAN, G. (1977) Intrinsic B cell defect in the immunoglobulin deficiency of chronic lymphatic leukemia. *Clin. Res.* **25**, 482A.

FERNANDEZ, L.A., MACSWEEN, J.M. & LANGLEY, G.R. (1977) T-cell function in untreated B-cell chronic lymphocytic leukemia. *Cancer*, **39**, 1168.

FU, S.M., CHIORAZZI, N., KUNKEL, H.G., HALPER, J.P. & HARRIS, S.R. (1978) Induction of *in vitro* differentiation and immunoglobulin synthesis of human leukemic B lymphocytes. *J. exp. Med.* **148**, 1570.

GREAVES, M.F. & JANOSSY, G. (1978) Patterns of gene expression and the cellular origins of human leukaemias. *Biochim. Biophys. Acta*, **516**, 193.

GUNZ, F.W. & ANGUS, H.B. (1965) Leukemia and cancer in the same patient. *Cancer*, **18**, 145.

HERSEY, P., EDWARDS, A. & EDWARDS, J. (1976) Characterization of mononuclear effector cells in human blood. *Clin. exp. Immunol.* **23**, 104.

KAPLAN, M.E. & CLARK, C. (1974) An improved rosetting assay for detection of human T lymphocytes. *J. Immunol. Methods*, **5**, 131.

KEIGHTLY, R.G., COOPER, M.D. & LAWTON, A.R. (1976) The T cell dependence of B cell differentiation induced by pokeweed mitogen. *J. Immunol.* **117**, 1538.

KUMAGAI, K., ABO, T., SEKIZAWA, T. & SASAKI, M. (1975) Studies of surface immunoglobulins on human B lymphocytes. *J. Immunol.* **115**, 982.

MILLER, D.G. & KARNOFSKY, D.A. (1961) Immunologic factors and resistance to infection in chronic lymphatic leukemia. *Am. J. Med.* **31**, 748.

MORETTA, L., WEBB, S.R., GROSSI, C.E., LYDYARD, D.M. & COOPER, M.D. (1977) Functional analysis of two human T-cell subpopulations. Help and suppression of B-cell responses by T-cells bearing receptors for IgM or IgG. *J. exp. Med.* **146**, 184.

SHAW, J.M., HAGON, E.E., VINCENT, P.C. & GUNZ, F.W. (1974) Quantitative studies on the response of normal and leukaemic lymphocytes to phytohaemagglutinin. *Aust. J. exp. Biol. Med. Sci.* **52**, 87.

WALDMANN, T.A., BRODER, S., BLAESE, R.M., DURM, M., BLACKMAN, M. & STROBER, W. (1974) Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinaemia. *Lancet*, ii, 609.

WELLS, J.V. & FUDENBERG, H.H. (1971) Metabolism of radio-iodinated IgG in patients with abnormal serum IgG levels. 1. Hypergammaglobulinaemia. 2. Hypogammaglobulinaemia. *Clin. exp. Immunol.* **9**, 761.

WYBRAN, J., CHANTLER, S. & FUDENBERG, H.H. (1973) Isolation of normal T cells in chronic lymphocytic leukaemia. *Lancet*, i, 126.