Clin. exp. Immunol. (1987) **70,** 182–191

Expression of functional interleukin 2 receptors on chronic lymphocytic leukaemia B lymphocytes is modulated by recombinant interleukin 2

J. J. MURPHY, V. MALKOVSKA*, L. HUDSON & R. E. MILLARD* *Departments of Immunology and Haematology*, St George's Hospital and Medical School, London*

(Accepted for publication 7 May 1987)

SUMMARY

In 16 of 18 chronic lymphocytic leukaemia (CLL) patients examined, a significant proportion of B cells in the leukaemic clone bound monoclonal antibodies specific for the interleukin 2 (IL-2) receptor site (CD25). B lymphocytes from patients tested showed a direct response to recombinant interleukin (rIL-2) during culture *in vitro* as shown by: (a) a ligand-mediated upregulation in the level of IL-2 receptor (IL-2R) expression (12 of 12 patients), (b) an increase in cell size (eight of nine patients), (c) an increase in ^{3}H-thymidine uptake (four of six patients). Taken together, this evidence suggests that the majority of leukaemic B cells from all the CLL patients examined expressed functional IL-2 receptors *in vitro*. Intriguingly, maximal receptor upregulation or increase in cell size was achieved at a lower concentration (50 u/ml) of rIL-2 than was required to achieve maximal ^{3}H-thymidine incorporation.

Keywords chronic lymphocytic leukaemia flow cytometry interleukin 2 receptors

INTRODUCTION

Most cases of chronic lymphocytic leukaemia (CLL) are characterized by a monoclonal expansion of B lymphocytes which, in the peripheral blood, appear frozen at a maturation stage intermediate between the pre-B cell and mature B lymphocyte (Johnstone, 1982; Preud'homme & Seligman, 1972). *In vitro,* however, these cells may be induced to synthesize DNA (Lantz *et al.*, 1985) and differentiate into immunoglobulin-secreting cells by a variety of mitogens (Guglielmi *et al.*, 1982; Totterman, Nillssom & Sundstrom, 1980) or T cell derived factors (Fu *et al.*, 1978; Yoshizaki *et al.*, 1982). Recently, interleukin 2 (IL-2) has been found to promote CLL B lymphocyte DNA synthesis and differentiation in the majority of CLL patients after pre-activation with anti-IgM antibody or *Staphylococcus aureus* Cowan strain I (Lantz *et al.*, 1985).

Armitage & Cawley (1986) reported finding IL-2 receptors on CLL cells and one of nine patients studied by Lantz and colleagues (1985) responded to IL-2 without preactivation. We have confirmed and extended these observations, using monoclonal antibodies and continuous flow microfluorimetry to quantify IL-2 receptor (IL-2R) site expression in CLL patients. Double antibody two-colour fluorescence showed definitively that, in the majority of CLL patients, a significant proportion of cells in the leukaemic clone expressed IL-2 receptors (CD25). Intriguingly, exposure of these cells to recombinant interleukin 2 (rIL-2) *in vitro* not only increased IL-2 receptor expression but also resulted in cell activation as shown by an increase in cell size and uptake of ^{3}H-thymidine.

Correspondence: John J. Murphy, Department of Immunology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK.

Table 1. Expression of IL-2 receptors on B lymphocytes from CLL patients

Patient	Rai stage	Lymphocyte count (10^{-9}/l)	% Tac positive lymphocytes	
			day 0	day 4
T.C.	0	7·8	8	ND
M.Ha.	0	14·8	2	ND
D.B.	0	18·4	4	ND
D.D.	0	20·3	15	ND
W.C.	0	21·0	0	ND
E.B.	0	26·0	19	ND
D.F.	0	31·4	9	52
W.N.	0	36·0	17	ND
D.Fd.	0	75·0	12	21
W.M.	0	98·9	0	22
A.B.	I	41·4	5	37
L.D.	II	52·0	20	30
M.S.	II	60·0	5	23
W.G.	II	78·0	9	ND
A.W.	II	145·0	50	ND
D.H.	III	ND	34	ND
G.S.	IV	49·0	12	ND
M.H.	IV	747·0	25	ND

The proportion of Tac (CD25) positive cells was determined on freshly isolated CLL B lymphocytes or after incubation in rIL-2 (500 u/ml) for 4 days using continuous flow microfluorimetry. T3 (CD3) and T11 (CD2) positive cells were $< 1\%$ in these experiments.

ND not determined.

MATERIALS AND METHODS

Patients. Eighteen well-documented cases of CLL were studied. Diagnosis was confirmed by cell surface marker studies and clinical staging (Table 1) was according to the Rai scheme (Rai *et al.*, 1975). All of the patients were untreated.

Isolation and characterisation of lymphocytes. Mononuclear cells were isolated from heparinized peripheral blood by centrifugation on a single step gradient of Ficoll-Hypaque (Lymphoprep, Nyegaard, Holland). T lymphocytes were depleted to $< 1{\cdot}0\%$ by two cycles of rosette cell formation with 2-aminoethylisothiouronium bromide-treated sheep erythrocytes (Saxon, Feldhaus & Robbins, 1976) followed by density gradient fractionation to recover the non-rosetting B lymphocytes. Samples of purified B lymphocytes were cultured either in 16 mm flat-bottomed 24-well plates (1 ml of 10^6/ml) (Costar, Cambridge, Mass., USA), or in flat-bottomed 96-well microculture plates (200 μl of 10^6/ml) (Nunc Gibco Europe Ltd, Uxbridge, Middlesex) either alone or with a range of rIL-2 concentrations (≤ 500 u/ml). Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 in air. Lymphocyte activation was assessed either by cell size analysis (see below) or by the uptake of ^{3}H-thymidine at the times shown in the text. In the latter case 1 μCi of ^{3}H-thymidine (TRA 120, 185 Gbq/mmol Amersham International Ltd, Amersham, England) was added 16 h before cell harvesting and sample processing for beta scintillation spectrometry.

The recombinant IL-2 used in these studies was a generous gift of Hoffman La Roche, Basel, Switzerland and Biogen, Geneva, Switzerland.

Continuous flow microfluorimetry. The size distribution of mononuclear cells before and after rIL-2 treatment was determined by forward angle light scatter (FALS) analysis using an EPICS V cytofluorimeter (Coulter Electronics Ltd, Hialeah, Florida). Monoclonal antibodies against Tac

(CD25, IgG_{2a}, a generous gift of Dr T. Waldmann, National Cancer Institute, Bethesday, MD), T3 (CD3, IgG_{2a}, Ortho Diagnostics Ltd, High Wycombe, England) or T11 (CD2, IgG_{2a}, Coulter Electronics Ltd, Luton, England) were detected in indirect immunofluorescent studies by a fluorescein isothiocyanate (FITC) conjugated goat anti-mouse immunoglobulin antibody (Coulter Electronics, Luton, England). Direct, double antibody two-colour fluorescence was carried out using a phycoerythrin (PE) conjugated monoclonal antibody against the IL-2 receptor site (CD25, IgG_1, Becton Dickenson, California) and an FITC conjugated anti-B1 antibody (CD20, IgG_{2a}, Coulter Electronics Ltd, Luton, England) or FITC conjugated goat anti-human immunoglobulin serum (Miles Labs. Ltd, Slough, England). Anti-B1 defines an antigen on human B cells with an approximate mol. wt of 35 kD (Stashenko *et al.*, 1980) and binds to a wide range of developing and mature B lymphocytes, as well as CLL B cells (Gordon *et al.*, 1983). In each case conjugated and unconjugated antibodies were used at their predetermined optimum dilution, and irrelevant mouse monoclonal antibodies of IgG_1 and IgG_{2a} isotypes were used as controls, to measure non-specific binding.

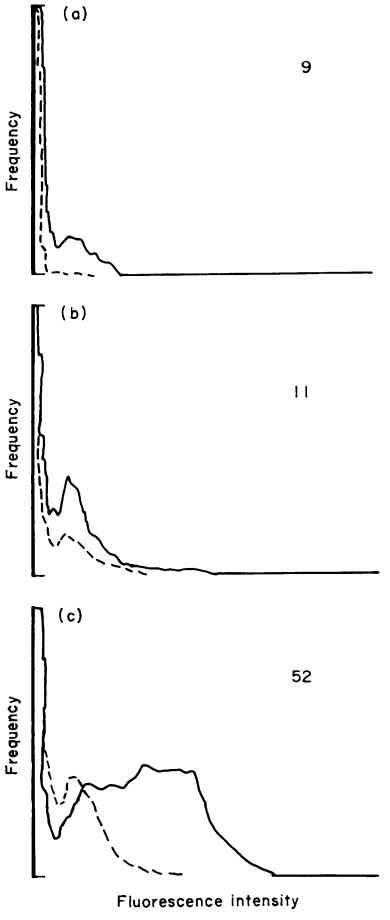

Fig. 1. Intensity profile of the binding of the anti-Tac monoclonal antibody to CLL B lymphocytes. (Patient D.F.). Horizontal scale depicts the distribution of log integral relative fluorescence intensity over 255 channels of indirect immunofluorescence with anti-Tac (continuous line) or an irrelevant antibody (broken line) on (a) CLL B lymphocytes at initial isolation, (b) after culture for 4 days in medium alone or (c) after culture for 4 days in rIL-2 (500 u/ml). Numbers show percent Tac positive cells above background.

For immunofluorescent labelling, 10^6 cells were washed by centrifugation (150 *g* for 10 min at 4°C) using ice-cold RPMI 1640 tissue culture medium containing 10 mM sodium azide and 1% fetal calf serum (staining solution). For both direct and indirect immunofluorescence, the cells were incubated with the appropriate antibody for 30 min on ice, followed by two washes in staining solution. Single antibody stained cells were resuspended in 0·5 ml of 0·5 mg/ml propidium iodide (Sigma Chemicals, Poole, England) in phosphate-buffered saline. Double antibody stained cells were resuspended in 0·5 ml of staining solution. All samples were analysed immediately.

The lymphocyte population in the suspensions was delimited by a combination of electronic gates set on size (FALS) and granularity (90° light scatter) and the distribution of fluorescence intensity in this 'gated-in' population determined on a logarithmic scale using an EPICS V. Owing to the high numbers of lymphocytes in the patients' blood, contaminating monocytes, clearly identifiable as a separate population using the FALS and 90° light scatter (Hoffman *et al.*, 1980), were $<0{\cdot}2\%$ in these experiments. For single antibody stained lymphocytes, dead cells were excluded from the antibody staining profiles by 'gating out' propidium iodide stained cells. During double-antibody two colour analysis, cell suspensions stained with FITC or PE labelled antibodies alone were used to correct the photodetector signals from the flow cytometer to ensure there was no breakthrough between the green and red channels.

Profiles of fluorescent staining were normalized by analysing 10^4 lymphocytes for each of the samples reported here.

RESULTS

In 16 of the 18 patients examined, a significant proportion of CLL B lymphocytes ($15{\cdot}4 \pm 12{\cdot}7\%$; day 0 Table 1 (mean $\pm$ s.d.)) expressed Tac antigen (CD25) before cultivation *in vitro* in the absence of detectable T3 (CD3) or T11 (CD2) positive cells (Table 1). Although there was no direct correlation between % Tac positive cells and Rai staging, B cells from stage 0 patients had the lowest proportion of positive cells (10/10 stage 0 patients $<20\%$, 4/8 patients stages I–IV $>20\%$). A typical distribution of the number of IL-2 receptors per cell in the B-CLL population (measured as log relative fluorescence intensity of Tac binding in 255 channels) is shown for one patient (D.F.) in Fig. 1. Upon initial isolation only 9% of cells were Tac positive with a low overall relative intensity range of 14–50 and a peak at channel 33 (Fig. 1a). Although incubation in medium alone for 4 days resulted in a small increase in the proportion and relative intensity of Tac bearing cells (11% positive cells, range 14–55 with peak at channel 38 (Fig. 1b)), this was insignificant compared to the increase in the level of Tac expression after incubation in rIL-2 (52% positive cells, 14–124 range with peak at channel 80 (Fig. 1c)). In each case the number of anti-Tac binding lymphocytes was determined by reference to cells treated wtih an irrelevant mouse monoclonal antibody (IgG_{2a}) and FITC conjugated anti-mouse immunoglobulin. In addition, non-specific staining from dead cells was excluded by 'gating out' propidium iodide stained cells. Very few dead cells were present: only 1·1% in the freshly isolated cells (Fig. 1a), 3·9% in cells incubated in medium alone (Fig. 1b), and 2·0% in cells incubated in rIL-2 (Fig. 1c). Following exposure to rIL-2 there was a significant increase in the mean cell size of CLL B cells ($n=9$, $P<0{\cdot}05$). The effect of rIL-2 concentration on Tac antigen (CD25) expression and cell size using CLL B cells was examined as shown in Fig. 2. In the majority of patients examined the maximum increase in IL-2 receptor density (Fig. 2a) or maximum increase in cell size (Fig. 2b) was obtained with 50 u/ml rIL-2 during 4 days of culture. Figure 3 shows the time course of the increase in Tac expression on cells from patient (W.M.). In this case, although no anti-Tac binding lymphocytes were seen upon lymphocyte isolation, incubation in rIL-2 resulted in a rapid increase both in the proportion of Tac positive cells (up to a maximum of 35% at day 3) and in the modal channel number for fluorescence intensity. Both parameters changed with virtually the same kinetics (full data not shown). Again, incubation in medium alone resulted in a small increase in the proportion of Tac positive cells (peak of 4·5% at day 3) but no change in the modal channel number for fluorescence intensity.

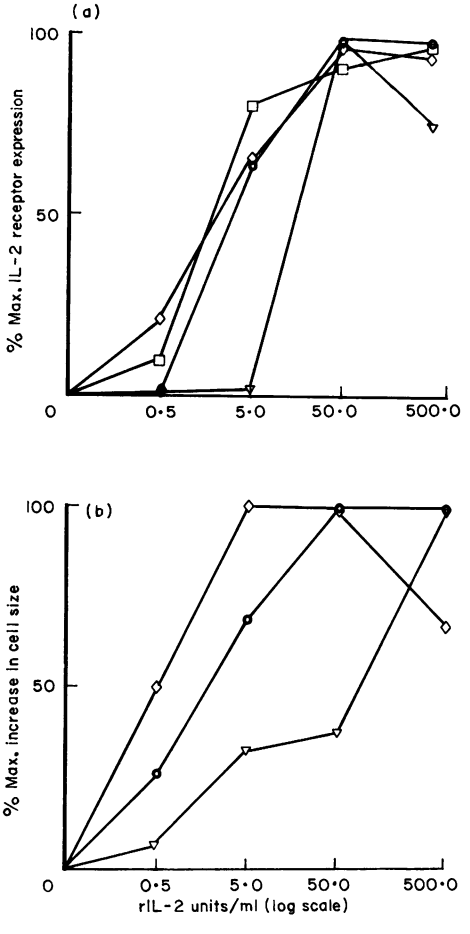

Fig. 2. Dose-dependent effects of rIL-2 on Tac (CD25) expression and cell size of CLL B lymphocytes. CLL B lymphocytes were incubated in different concentrations of rIL-2 for 4 days: (a) dose-dependent effect of rIL-2 on Tac expression from cells of four patients: A.B. (▽), D.F. (○), D.Fd. (◇) and M.S. (□) (mean channel number ± s.d. for log fluorescence profile at maximum IL-2 receptor expression was 61·0 ± 10·5 ($n = 4$)) and (b) dose-dependent increase in cell size using cells from three of the above patients as measured by forward angle light scatter (mean channel number ± s.d. for forward angle light scatter profile at maximum increases in cell size was 148·3 ± 28·1 ($n = 3$)).

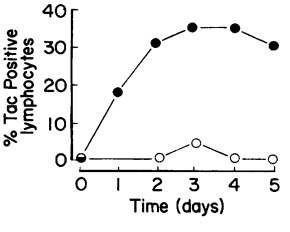

Fig. 3. Time course of increase in anti-Tac binding following incubation in rIL-2. B lymphocytes from a CLL patient (W.M.) were incubated either in medium alone (open symbols) or with rIL-2 (500 u/ml), (closed symbols) for up to 5 days at 37°C. At each time point samples of cells were stained with anti-Tac by indirect immunofluorescence, and binding quantified by continuous flow microfluorimetry. Data from one experiment; two other experiments from different patients gave similar results.

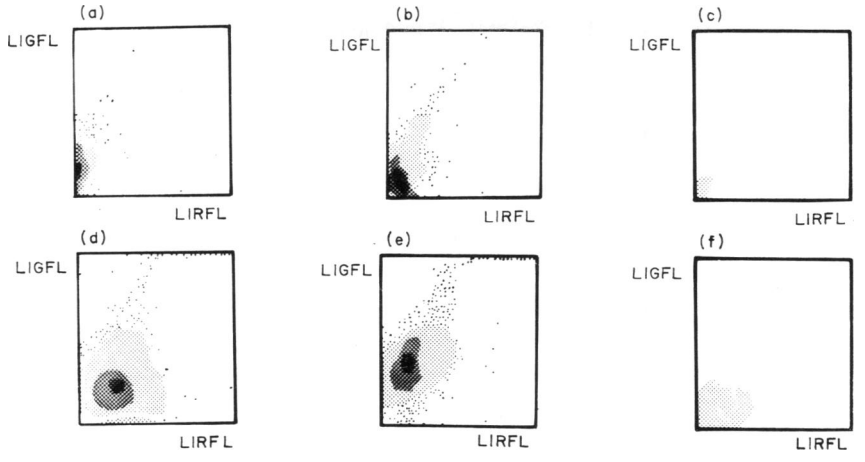

Fig. 4. Distribution of IL-2 receptors on CLL B lymphocytes determined by double immunofluorescence (Patient W.N.). CLL B lymphocytes were either examined (a, c) immediately after isolation, (b) after incubation for 4 days in medium alone or (d, e and f) after incubation in rIL-2. The B cells were identified by FITC conjugated anti-B1 monoclonal (CD20) (a, b, d) or by FITC conjugated anti-human immunoglobulin (e), and their IL-2 receptor expression quantified by phycoerythrin conjugated anti-IL-2R antibody (CD25). Non-specific staining patterns with irrelevant isotype matched mouse antibodies on cells at initial isolation (c) and after 4 days culture in rIL-2 (f) are also shown. Data shown as plan view of two parameter histograms of log integral green fluorescence of FITC conjugated anti-B1 (LIGFL) versus log integral red fluorescence of PE conjugated anti-IL 2R (LIRFL) sliced at 4, 40 and 400 events per channel (corresponding to increase in density of stipling). The results of six experiments in which the proportion of IL-2R and B1 positive cells were monitored before and after exposure to rIL-2 are shown below. All patients were Rai stage 0.

	Percent lymphocytes carrying IL-2R and B1 marker	
Patient	Day 0	Day 4
D.B.	4	9
T.C.	8	37
D.D.	15	81
M.Ha.	8	39
W.M.	6	21
W.N.	2	69

The effect of incubation in rIL-2 on IL-2 receptor expression was determined for six patients under optimum conditions (Table 1). In each case, incubation in rIL-2 for 4 days resulted in an increase in the proportion of CLL B cells expressing IL-2 receptors.

Staining with T3 (CD3) or T11 (CD2) antibodies was used to determine the proportion of T cells in the B cell cultures at the start and end of the culture experiments. In each of the experiments reported here, T cell contamination was never greater than 1% at any stage. Nevertheless, to confirm that the increase in IL-2 receptor expression was associated with the leukaemic clone, double antibody two colour fluorescence analysis was carried out. B cells were defined by an FITC conjugated B1 (CD20) or anti-Ig antibody and IL-2 receptors detected by the PE conjugated anti-IL-2R monoclonal (CD25) (Fig. 4). When CLL B cells were freshly isolated from patient W.N., only 2% of cells were IL-2R positive and B1 positive (Fig. 4a). The proportion of double positive cells reached 10% after incubation for 4 days in medium alone (Fig. 4b). However, in both cases the

Table 2. Effect of rIL-2 on ^{3}H-thymidine uptake of CLL B lymphocytes

Patient	Rai Stage	^{3}H-thymidine incorporation (ct/min) after incubation in	
		Medium alone	rIL-2
D.F.	0	65±0·20	5326±0·01
Df.D.	0	51±0·13	949±0·10
W.N.	0	166±0·14	5001±0·04
A.B.	I	50±0·20	1812±0·13
L.D.	II	31±0·01	46±0·18
M.S.	II	38±0·01	43±0·10

T depleted CLL B lymphocytes were incubated in medium alone or in medium and rIL-2 (500 u/ml) for 4 days. ^{3}H-thymidine uptake was measured over the last 16 h of the experiment. Data shown as geometric mean±log. s.d. for three determinations.

fluorescence intensity profile was restricted to low channel numbers. When cells from the same pool were incubated in rIL-2, 69% expressed both the IL-2R and B1 markers (Fig. 4d) and a similar proportion (62%) of IL-2R bearing B cells was seen with the anti-Ig marker (Fig. 4e). Figures 4c and 4f show non-specific staining, using the isotype matched irrelevant mouse antibodies on freshly isolated cells, and cells after 4 days incubation with rIL-2, respectively.

The effect of rIL-2 on ^{3}H-thymidine uptake was tested for on CLL B cells from six patients and the results are summarized in Table 2. B cells from four patients (A.B., D.F., D.Fd., W.N.) showed significant increases in ^{3}H-thymidine uptake upon exposure to rIL-2 (500 u/ml) while two failed to respond (L.D., M.S.).

DISCUSSION

The expression of IL-2 receptors was originally considered to be an activation marker exclusive for T lymphocytes (Uchiyama, Broder & Waldmann, 1981). However, recent studies have demonstrated identical receptors on normal human B lymphocytes, but only after activation *in vitro* with mitogens or T cell factors (Jung, Hara & Fu, 1984; Mingari *et al.*, 1984; Muraguchi *et al.*, 1985; Suzuki & Cooper, 1985; Tsudo, Uchiyama & Uchino, 1984; Waldmann *et al.*, 1984). Consequently, the description of IL-2 receptors on freshly isolated large B lymphocytes (Suzuki & Cooper, 1985), hairy cell leukaemia B cells (Korsmeyer *et al.*, 1983), EBV transformed B lines from Burkitt lymphoma patients (Waldmann *et al.*, 1984) and CLL B cells (Armitage & Cawley, 1986) has been taken as evidence that these normal or transformed cells express an activated phenotype *in vivo*. This view is further reinforced for CLL B cells by the detection of other activation markers including B-LAST 1 (Thorley-Lawson *et al.*, 1982) and MHM 6 (J. Gordon, pers. comm.) on these cells immediately after isolation.

In the present study we have quantified IL-2 receptors on CLL B cells using two different monoclonal antibodies and flow cytometry. It seems likely from our data, and those already published (Armitage & Cawley, 1986), that all CLL B cells express IL-2 receptors, albeit at low levels. This would explain the uniform responsiveness of CLL patients B cells to rIL-2 treatment *in vitro* even though some of these cells failed to bind the anti-Tac monoclonal antibody on initial isolation (for example, patient W.M., Fig. 3). Although it is not possible to exclude the effects of mediators produced from contaminating T lymphocytes or monocytes stimulated by rIL-2, we suspect that the IL-2 acted directly on CLL B cells themselves without the need for T cells or T cell

products. This view is supported by our observation that rIL-2 receptor regulation, increases in cell size and ^{3}H-thymidine uptake occurred when much less than 1% T3 (CD3) cells were present in B cell populations. Similarly, the double immunofluorescence studies using markers from leukaemic cells and the anti-IL-2R antibody (Fig. 4) showed unequivocally that the IL 2 receptors were indeed expressed on B cells.

It is probable that both of the monoclonal antibodies used to detect the IL-2 receptor bind equally well to high and low affinity receptor sites (Smith & Cantrell, 1985). Recently a 75 kD IL-2 binding protein has been described that does not bind the Tac antibody (Teshigawara *et al.*, 1987). It has been proposed that expression of this protein and the 55 kD Tac binding protein together are necessary for high affinity IL-2 receptor expression (Smith, 1987). We do not know whether direct stimulation with rIL-2 in our system eventually leads to high affinity receptor expression. IL-2 receptor expression and cell size increases of CLL B cells may well be influenced by IL-2 at physiological concentrations but higher concentrations ($\geqq$250/ml) were needed for maximum increases in ^{3}H-thymidine uptake (Malkovska *et al.*, 1987). It may be that receptor upregulation and increases in cell size can be mediated by interaction of IL-2 with low affinity receptors whereas induction of DNA synthesis requires interaction of IL-2 with high affinity receptors. It is, however, recognized that CLL B cells show poor proliferative responses (Godal & Funderud, 1982) and IL-2 is probably only one of a number of growth factors which influence B cell growth and differentiation (Romagnani *et al.*, 1986a; Jelinek, Splanski & Lipsky, 1986). Consequently, it is perhaps not surprising that very high concentrations of IL-2 were needed for the induction of relatively small amounts of DNA synthesis.

The rate of increase in the number of IL-2 receptor positive cells after rIL-2 stimulation (Fig. 3) is consistent with upregulation of Tac antigen expression rather than an outgrowth of a minor Tac positive subpopulation. This explanation is reinforced by recent observations showing that CLL B lymphocytes require at least 4 days exposure to rIL-2 before significant ^{3}H-thymidine uptake can be detected (Malkovska *et al.*, 1987), whereas Tac antigen expression was maximum after only 3 days of culture (Fig. 3). It should be stressed that the effects observed on the CLL B cells are thought to be IL-2 regulated and are not due to trace contaminants in the rIL-2 preparation, for example, endotoxin lipopolysaccharide. The recombinant IL-2 used was purified to biochemical homogeneity by reverse phase chromatography and two independent sources gave identical results which titrated according to the IL-2 units of activity. In addition, Romagnani and colleagues (1986b) have shown that the effects of Biogen rIL-2 can be blocked by anti-IL-2 receptor antibody. Finally, lipopolysaccharide was not detected in this preparation of rIL-2 by the rabbit pyrogen test or the Limulus amoebocyte test (Liang *et al.*, 1985).

The general phenomenon of receptor regulation by exposure to its specific ligand is well known. For example, the insulin receptor on 3T3 cells shows increased expression after contact with insulin (King, Rechler & Kahn, 1982). If IL-2 receptor expression is indeed an activation marker then it is not clear whether its presence on CLL B cells is as a result of activation *in vivo* via a 'physiological route' or as a consequence of a transformed cell phenotype. The low but significant increase in Tac positivity following cultivation *in vitro* without deliberate stimulation might be consequential on stimulation *in vitro* or an unrecognized effect of the culture conditions. Intriguingly, whatever the significance of IL-2 receptor expression on CLL cells, the receptors are functional as evidenced by the increase in cell size (Fig. 2b) and ^{3}H-thymidine uptake (Table 2), as well as the regulation of receptor expression (Table 1, Fig. 4) following exposure to rIL-2.

If, as was suggested earlier (Lantz *et al.*, 1985) IL-2 might be responsible for the expansion of cells in CLL, then the original target cells must be much more responsive to IL-2 *in vivo* than the lymphocytes recovered from the peripheral blood in view of the unphysiological amounts of rIL-2 required to cause modest increases in ^{3}H-thymidine uptake. Only cells from Rai stage O or I patients responded to rIL-2 with increased ^{3}H-thymidine uptake in the present study. It is not possible, however, to assess the significance of this observation due to the limited numbers of patients tested. Nevertheless, it has been shown that CLL B cell IL-2 receptor expression and cell size are modulated by physiological amounts of IL-2 and this is further evidence for a possible role of IL-2 in this disease.

This work was supported by grants from the Cancer Research Campaign of Great Britain. We are extremely grateful to Professor P. Flute and Drs J. Parker-Williams and D. Bevan for their clinical collaboration in this study. Thanks are due to Gurdip Kour for excellent technical assistance and to Mrs Megan Evans for preparing the manuscript.

REFERENCES

ARMITAGE, R.J. & CAWLEY, T.C. (1986) Normal and certain leukaemic B cells express IL-2 receptors without *in vitro* activation. *Clin. exp. Immunol.* **63,** 298.

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 leukaemic B lymphocytes. *J. exp. Med.* **148,** 1570.

GODAL, T. & FUNDERUD, S. (1982) Human B-cell neoplasms in relation to normal B-cell differentiation and maturation processes. *Adv. Cancer Res.* **36,** 211.

GORDON, J., MELLSTEDT, H., AMAN, P., BIBERFELD, P., BJORHOLM, M. & KLEIN, G. (1983) Phenotypes in chronic B-lymphocytic leukemia probed by monoclonal antibodies and immunoglobulin secretion studies: identification of stages of maturation arrest and the relation to clinical findings. *Blood* **62,** 910.

GUGLIELMI, P., PREUD'HOMME, J.L., CIORBARU-BAROT, R. & SELIGMANN, M. (1982) Mitogen-induced maturation of chronic lymphocytic leukaemia B lymphocytes. *J. clin. Immunol.* **2,** 186.

HOFFMAN, R.A., KUNG, P.C., HANSEN, W.P. & GOLDSTEIN, G. (1980) Simple and rapid measurement of human T lymphocytes and their subclasses in peripheral blood. *Proc. natn. Acad. Sci. USA.* **77,** 4914.

JELINEK, D.F., SPLANSKI, J.B. & LIPSKY, P.E. (1986) The roles of interleukin 2 and interferon-γ in B cell activation, growth and differentiation. *Eur. J. Immunol.* **16,** 925.

JOHNSTONE, A.P. (1982) Chronic lymphocytic leukaemia and its relationship to normal lymphopoiesis. *Immunol. Today* **3,** 343.

JUNG, L.K.L., HARA, T. & FU, S.M. (1984) Detection and functional studies of P60-65 (Tac antibody) on activated human B cells. *J. exp. Med.* **160,** 1547.

KING, G.L., RECHLER, M.M. & KAHN, C.R. (1982) Interaction between the receptors for insulin and the insulin-like growth factors on adipocytes. *J. biol. Chem.* **257,** 1001.

KORSMEYER, S.J., GREENE, W.C., COSSMAN, J., HSU, S.M., JENSEN, J.P., NECKERS, L.M., MARSHALL, S.L., BAKHSHI, A., DEPPER, J.M., LEONARD, W.J., JAFFE, E.S. & WALDMANN, T.A. (1983) Rearrangement and expression of immunoglobulin genes and expression of Tac antigen in Hairy Cell Leukaemia. *Proc. natn. Acad. Sci. USA.* **80,** 4522.

LANTZ, O., GRILLOT-COURVALIN, C., SCHMITT, C., FERMAND, J. & BROUET, J. (1985) Interleukin 2-induced proliferation of leukemic human B cells. *J. exp. Med.* **161,** 1225.

LIANG, S., ALLET, B., ROSE, K., HIRSCHI, M., LIANG, C. & THATCHER, D.R. (1985) Characterisation of human interleukin 2 derived from *Escherichia coli*. *Biochem. J.* **229,** 429.

MALKOVSKA, V., MURPHY, J., HUDSON, L. & BEVAN, D. (1987) Direct effect of interleukin 2 on chronic lymphocytic leukaemia B cell functions and morphology. *Clin. exp. Immunol.* **68,** 677.

MINGARI, M.C., GEROSA, F., CARDA, G., ACCOLLA, R.S., MORETTA, A., ZUBLER, R.A., WALDMANN, T.A. & MORETTA, L. (1984) Human interleukin-2 promotes proliferation of activated B cells via surface receptors similar to those of activated T cells. *Nature (Lond.)*, **310,** 640.

MARAGUCHI, A., KEHRL, J.H., LONGO, D.L., VOLKMAN, D.J., SMITH, K.A. & FAUCI, A.S. (1985) Interleukin 2 receptors on human B cells. Implications for the role of interleukin 2 in human B cell function. *J. exp. Med.* **161,** 181.

PREUD'HOMME, J.L. & SELIGMANN, M. (1972) Surface bound immunoglobulins as a cell marker in human lymphoproliferative diseases. *Blood* **40,** 777.

RAI, K.R., SAWITSKY, A., CRONKITE, E.P., CHANANA, A.D., LEVY, R.N. & PASTERNAK, B.S. (1975) Clinical staging of chronic lymphocytic leukaemia. *Blood* **46,** 219.

ROMAGNANI, S., GIUDIZI, G.M., ALMERIGOGNA, F., BIAGIOTTI, R., ALESSI, A., MINGARI, C., LIANG, C.-M., MORETTA, L. & RICCI, M. (1986a) Analysis of the role of interferon-gamma, interleukin 2 and a third factor distinct from interferon-gamma and interleukin 2 on human B cell proliferation. Evidence that they act at different times after B cell activation. *Eur. J. Immunol.* **16,** 623.

ROMAGNANI, S., DEL PRETE, G., GIUDIZI, M.G., BIAGOTTA, R., ALMERIGOGNA, F., TIRI, A., ALESSI, A., MAZETTI, M. & RICCI, M. (1986b) Direct induction of human B-cell differentiation by recombinant interleukin-2. *Immunology* **58,** 31.

SAXON, A., FELDHAUS, J. & ROBBINS, R.A. (1976) Single-step separations of human T and B cells using AET-treated SRBC rosettes. *J. immunol. Methods.* **12,** 285.

SMITH, K.A. (1987) The two chain structure of high-affinity IL-2 receptors. *Immunology Today* **8,** 11.

SMITH, K.A. & CANTRELL, D.A. (1985) Interleukin 2 regulates its own receptors. *Proc. natn. Acad. Sci. USA.* **82,** 864.

STASHENKO, P., NADLER, L.M., HARDY, R. & SCHLOSSMAN, S.F. (1980) Characterisation of a human B cell specific antigen. *J. Immunol.* **125,** 1678.

SUZUKI, T. & COOPER, M.D. (1985) Comparison of the expression of IL2 receptors by human T and B cells: induction by the polyclonal mitogens, phorbol myristate acetate, and anti-mu antibody. *J. Immunol.* **134,** 3111.

TESHIGAWARA, K., WANG, H-M., KATO, K. & SMITH, K.A. (1987) Interleukin 2 high-affinity receptor expression requires two distinct binding proteins. *J. exp. Med.* **165,** 223.

THORLEY LAWSON, D.A., SCHOOLEY, R.T., BHAN, A.K. & NADLER, L.M. (1982) Epstein-Barr virus superinduces a new human B cell differentiation antigen (B-LAST 1) expressed on transformed lymphoblasts. *Cell* **30,** 415.

TOTTERMAN, T.H., NILSSOM, K. & SUNDSTROM, C. (1980) Phorbol-ester induced differentiation of chronic lymphocytic leukaemia cells. *Nature (Lond.)*, **228,** 176.

TSUDO, M., UCHIYAMA, T. & UCHINO, H. (1984) Expression of Tac antigen on activated normal human B cells. *J. exp. Med.* **160,** 612.

UCHIYAMA, T., BRODER, S. & WALDMANN, T.A. (1981) A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. 1. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J. Immunol.* **126,** 1393.

WALDMANN, T.A., GOLDMAN, C.K., LEONARD, W.J., PEPPER, J.M., ROBB, R.J., KORSMEYER, S.J. & GREENE, W.C. (1984) Interleukin-2 receptors on activated malignant and normal B cells. *Curr. Topics Microbiol. Immunol.* **113,** 96.

YOSHIZAKI, K., NAKAGAWA, T., KAIDEA, T., MURAGUCHI, A., YAMAMURA, Y. & KISHIMOTO, T. (1982) Induction of proliferation and Ig production in human B leukemic cells by anti-immunoglobulins and T cell factors. *J. Immunol.* **128,** 1296.