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# Cellular activation without proliferation to B cell growth factor and interleukin 2 in chronic lymphocytic leukaemia B cells stimulated with phorbol ester plus calcium ionophore

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## SUMMARY

Individual leukaemic B cells of chronic lymphocytic leukaemia (CLL) do not proliferate to B cell growth factor (BCGF) or interleukin 2 (IL-2) when co-stimulated with immunoglobulin (Ig) ligands. To exclude possible defective signalling via surface Ig (sIg), phorbol myristate acetate (PMA) plus calcium ionophore (A23187) were used to activate purified CLL B cells and compared with staphylococcal protein A coupled to sepharose beads (Seph-PA). RNA synthesis and phenotypic changes after PMA plus A23187 stimulation indicate that CLL B cells from (10) different individuals are similarly able to undergo the $G_0$ to the $G_1$ phase transition and express surface activation antigens. In contrast, they are variable in the capacity to show DNA synthesis, which occurred in only six out of 10 cases. Even in the presence of BCGF (10%, v/v) or IL-2 (50 U/ml) four out of nine CLL B cells activated with PMA plus A23187 or PMA alone were still unable to proliferate although they were induced to express CD23, 4F2, CD25 and OKT9 antigens by PMA plus A23187. However, PMA plus A23187 induced IgM secretion which increased further in response to IL-2 even in the absence of DNA synthesis. Moreover, in other CLL B cell populations, the unresponsiveness to growth factors upon co-stimulation with Ig ligands (Seph-PA) may simply reflect a defective signalling via sIg cross-linking which can be circumvented by PMA plus A23187 stimulation. Recombinant Interferon-$\gamma$ (50 U/ml) failed to affect DNA synthesis and IgM secretion.


**Keywords** CLL B cell activation phorbol ester calcium ionophore BCGF IL-2

## INTRODUCTION

Despite their phenotypic similarity (Catovsky *et al.*, 1979; Anderson *et al.*, 1984), different leukaemic B cell populations of chronic lymphocytic leukaemia (CLL) are variable in their proliferative responsiveness to B cell growth factor (BCGF) and interleukin 2 (IL-2) and many of them do not proliferate to these growth factors when co-stimulated with ligands of Ig such as anti-Ig antibodies or Staphylococcus aureus Cowan I (SAC) (Hivroz *et al.*, 1986; Perri, 1986; Karray *et al.*, 1987). The reasons for this variability and unresponsiveness are unknown.

Capping of surface Ig (sIg) fails to occur or is defective in CLL B cell populations (Cohen, 1975), and most do not proliferate when stimulated with anti-Ig antibodies or SAC (Tamaki *et al.*, 1986). Thus, in co-stimulatory assays with ligands of Ig, the unresponsiveness to growth factors might simply reflect that Ig cross-linking fails to activate these cells or, alternatively, this can reflect other particular features of the biology of these monoclonal B cells.

To gain information about these possibilities, in this study we have used phorbol myristate acetate (PMA) plus calcium ionophore, A23187, to activate CLL B cells and have compared their effectiveness to that of staphylococcal protein A coupled to sepharose beads (Seph-PA), a highly efficient sIg cross-linker (Forsgren, Svedjelund & Wigzell, 1976). This approach relies on the fact that PMA and A23187 act in synergy to activate normal B cells bypassing the need for the ligation of sIg (Clevers *et al.*, 1985; Guy *et al.*, 1985; Roifman *et al.*, 1987). Moreover, we have found previously that PMA plus A23187 induces some activation-associated events and DNA synthesis in both normal tonsil B cells and leukaemic B cells of some CLL patients (Engel *et al.*, 1987). However, detailed data on the capacity of these compounds to activate CLL B cells and to render them responsive to growth and differentiation factors were lacking.

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## MATERIALS AND METHODS

*Monoclonal antibodies*

Monoclonal antibodies (MoAb) CD22 (HD39) and CD23 (MHM6) were kindly provided by the 3rd Workshop on Human Leukocyte Differentiation Antigens (Dr McMichael, Oxford). FG 1/8 MoAb, recognizing the 4F2 activation antigen (Kehrl, Muruguchi & Fauci, 1984), was donated by Dr Sanchez-Madrid

**Table 1.** Cell surface marker phenotype of purified B cells from 10 CLL patients

| | Cell surface markers* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CLL B cells | CD19 | CD20 | CD21 | CD22 | CD24 | CD5 | Mr | sIg | CD23 | 4F2 | CD25 | OKT9 | HLA Class II |
| 1 | ++ | + | + | + | ++ | ++ | ++ | ++ | + | − | − | − | ++ |
| 2 | ++ | ++ | + | + | ++ | +† | ++ | ++ | + | − | −§ | − | ++ |
| 3 | + | ++ | + | + | ++ | ++ | ++ | ++ | + | − | − | − | ++ |
| 4 | ++ | ++ | ++ | + | ++ | ++ | ++ | ++ | − | + | − | − | ++ |
| 5 | ++ | ++ | + | + | ++ | ++ | ++ | ++ | ++ | + | − | − | ++ |
| 6 | ++ | ++ | ++ | + | ++ | +† | ++ | ++ | + | + | − | − | ++ |
| 7 | + | ++ | ++ | + | ++ | ++ | ++ | ++ | ++ | − | − | − | ++ |
| 8 | ++ | ++ | ++ | + | ++ | +† | ++ | ++ | + | − | −¶ | − | ++ |
| 9 | ++ | + | + | − | ++ | ++ | ++ | ++ | + | − | − | − | ++ |
| 10 | ++ | + | + | + | ++ | +† | ++ | ++ | −‡ | − | − | − | ++ |

* Percentage of positive cells: (++), > 50%; (+), 10-50%; (−). < 10%. In all cases, $CD3^+$ and $CD14^+$ cells were below 1%, and more than 96% expressed low intensity of sIg with either κ (eight cases) or λ (two cases) light chains.

† Indicates 37–50% of $CD5^+$ cells.

‡ Indicates 6% of $CD23^+$ cells.

§ Indicates 4% of $CD25^+$ cells.

¶ Indicates 8% of $CD25^+$ cells.

(Madrid). CD5(Cris-1), CD20(B-Cl), CD3(Cris-7) and CD14(Cris 6) MoAb, produced in our laboratory, have been clustered in the 1st, 2nd and 3rd International Workshops on Human Leukocyte Differentiation Antigens. EDU-1 MoAb, recognizing non-polymorphic HLA-Class II antigens and B-C2, a CD24 MoAb, were also produced locally (Anegón *et al.*, 1986; Engel *et al.*, 1987). MoAb CD19(B4), CD21(B2) (Coulter Clone, FL), CD25(IL-2R) (Becton Dickinson, Mountain View, CA) and OKT9 (Ortho Diagnostic Systems, Raritan, NJ) were also utilized.

*Lymphokines and reagents*

Purified 12 kD BCGF (Cellular Products Inc., Buffalo, NY) was used at a final concentration of 10% (v/v). Recombinant IL-2 (rIL-2) (Boeringer Mannheim GmbH, FRG) and recombinant Interferon-γ (rINF-γ) (Genetech, San Francisco, CA) were used at 50 U/ml final concentration. Phorbol 12 myristate 13 acetate (PMA) and calcium ionophore A23187 (Sigma Chemical Co, St. Louis, MO) were stored at −20°C diluted in ethanol at 1 mg/ml. Final concentrations in the cultures were 10 ng/ml for PMA, and 500 nM for A23187, which have been found appropriate to induce optimal activation of normal B cells (Clevers *et al.*, 1985; Engel *et al.*, 1987) and CLL B cells (Engel *et al.*, 1987). Seph-PA (Pharmacia Uppsala, Sweden) was used at a final concentraton of 50 μg/ml. Bovine serum albumin (Merck, Darmstadt, GFR) was coupled to CNBr-activated Sepharose 4B (Pharmacia) (Seph-BSA) and used as the control of Seph-PA.

*CLL B cell purification*

Leukaemic B cells from 10 CLL patients were studied. None of the patients was on therapy at the time of study. Mononuclear cells were isolated from heparinized peripheral blood by centrifugation on Ficoll-Hypaque gradients. T lymphocytes and monocytes were eliminated by two rounds of complement-mediated lysis with CD3(Cris-7) and CD14(Cris-6) MoAb using non-toxic fresh rabbit serum as the source of complement.

*Cell surface marker analysis*

Cell surface antigens detected by MoAb and sIg were analysed before and after in-vitro stimulation using an indirect immunofluorescence technique, already described (Anegón *et al.*, 1986). FITC-conjugated anti-mouse Ig goat antibodies (Sigma, St. Louis, MO), unconjugated goat antibodies specific for human Ig heavy and light chains (Tago Inc., Burlingame, CA) and FITC-conjugated anti-goat Ig swine antibodies (Tago Inc., Burlingame, CA) were utilized. Electronic cell volume and positive fluorescent cells were assessed by means of a FACS Analyzer (Becton Dickinson, Mountain View, CA). Rosette formation with mouse eythrocytes was also performed.

*Cell cultures and RNA and DNA synthesis*

Culture medium RPMI 1640 (Eurobio, Paris, France), supplemented with 10% Fetal calf serum (FCS) (Eurobio) and 2 mM of L-glutamine (Flow, UK) and containing 50 μg/ml of gentamicine, was used. Cultures were incubated at 37°C in a 100% humidified atmosphere of air/5% $CO_2$. Macrocultures ($2 \times 10^6$ cells/ml) were performed in 25 ml tissue culture flasks (Nunc, Roskilde, Denmark). Viable cultured cells were isolated by centrifugation over a Ficoll gradient, washed three times with culture medium and analysed for cell surface markers. Triplicate microcultures were performed ($2 \times 10^5$ cells/microwell) in flat-bottomed 96-well plates (Nunc, Roskilde, Denmark), in a final volume of 0·2 ml; RNA and DNA synthesis was evaluated by measuring the uptake (1 μCi/microwell) of $^3$H-uridine and $^3$H-thymidine (Amersham, UK), respectively, during the last 16–18 h of culture periods. Both one- and two-step microcultures were used to assess the responsiveness to lymphokines induced by PMA and PMA plus A23187. In the two-step microcultures cells were cultured for 3 days with or without these stimulators, in the absence or presence of each one of the lymphokines. After centrifugation of culture plates supernatants were discarded, microwells replenished with either culture medium or the corresponding lymphokine, and cells recultured for 3 additional

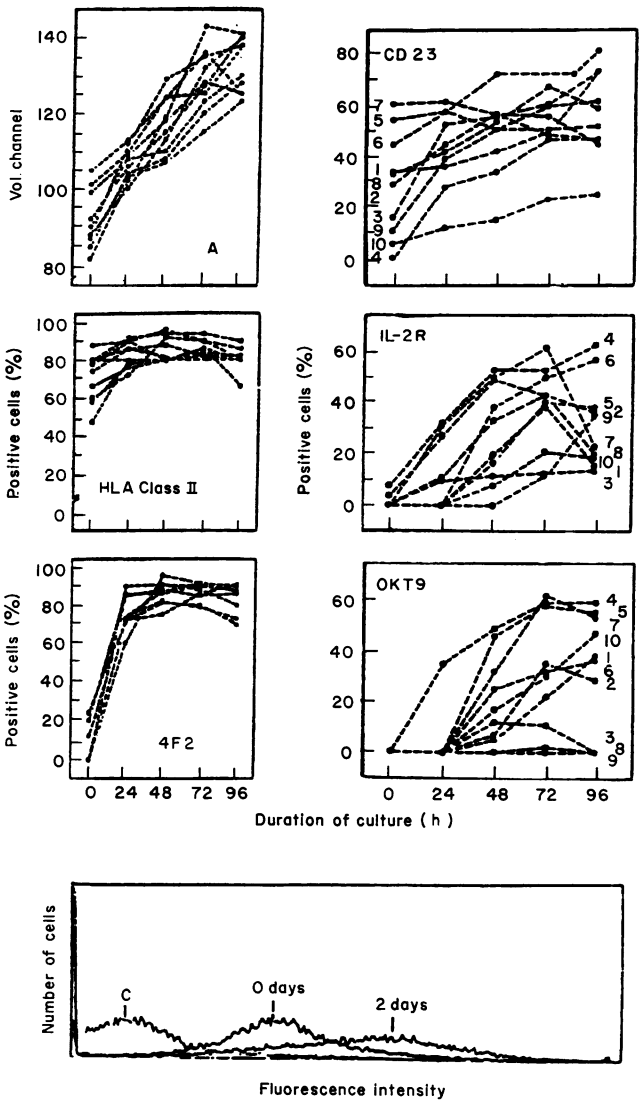


**Fig. 1.** Phenotypic changes induced by PMA plus A23187 in 10 CLL B cell populations. (Top) Cell volume (A), and number of cells positive for HLA-class II, 4F2, CD23, CD25 (IL-2R) and OKT9 antigens before and after 1–4 days of culture (Numbers within the plots identify CLL B cells). (Bottom) A representative FACS-Analyzer histogram of the expression intensity of HLA Class II antigens, before and after 2 days of culture, (C) indicates control staining with an unreactive MoAb.

days. DNA synthesis was evaluated at the end of the first and second culture steps, and IgM and light chain type were determined in the supernatants of the second culture step.

*IgM and light chain type determination in culture supernatants*
A sensitive ELISA was used which permits detection of 10 ng/ml of IgM (Gallart *et al.*, 1985). Duplicate determinations were performed in two different dilutions of pooled supernatants of triplicate microcultures.

## RESULTS

*Cell surface marker phenotype of purified CLL B cell populations*
$CD3^+$ and $CD14^+$ cells were $<1\%$ and $>96\%$ of cells showed low intensity of sIg. Co-expression of sIgM and sIgD was observed except in one case where only sIgM was found. Purified B cells were predominantly monoclonal CLL B cells as indicated by the fact that, in all cases, $\geqslant 93\%$ of cells expressed either $\kappa$ (eight cases) or $\lambda$ (two cases) light chains on their surface. Data on Mr formation and expression of CD5 and other cell surface antigens are shown in Table 1.

*Phenotypic changes induced by PMA plus A23187 in CLL B cell populations*
After 24 h of culture, most cultured cells from 10 CLL B cell samples expressed 4F2 antigen and during the following 3 days of culture, $4F2^+$ cells reached 83–96% (Fig. 1). In all cases cell volume enlargement and strong increase of HLA Class II antigen expression were observed during the 4-day culture period. As found by others (Thorley-Lawson *et al.*, 1985), the CD23 antigen, which is related to the BCGF receptors (reviewed in Gordon & Guy, 1987), was already expressed on most fresh CLL B cell populations. The number of $CD23^+$ cells increased during the 4 days of culture, although slightly as fresh CLL B cell samples already showed high numbers of $CD23^+$ cells. $CD25^+$ and $OKT9^+$ cells were detected after 24–48 h of culture and peaked on days 3 or 4 (Fig. 1). In samples no. 8 and 9, $OKT9^+$ cells were not detectable. In all cases the number of $CD3^+$ and $CD14^+$ cells remained below 1% or were undetectable (data not shown).

*RNA and DNA synthesis by CLL B cells*
After 40 h of culture with PMA plus A23187 significant, or very high levels of RNA synthesis were observed in all cases. After 3 and/or 6 days of culture with these compounds, consistent levels of DNA synthesis occurred in only two samples (nos. 4 and 10); in the remaining samples DNA synthesis was poor but significant (nos. 1, 7, 8 and 9) or absent (nos. 2, 3, 5 and 6). A23187 alone did not induce RNA and DNA synthesis but acted in synergy with PMA to increase RNA synthesis in most cases, and it also increased the PMA-induced DNA synthesis in cells no. 4, 8 and 9 (Table 2).

RNA and DNA synthesis after stimulation with Seph-PA was also evaluated in eight samples (nos. 1–8). RNA synthesis after 40 h of culture was observed in only two cases: no. 1 ($8928 \pm 79$ ct/min vs $3450 \pm 30$ ct/min with Seph-BSA control) and no. 4 ($81\,303 \pm 700$ ct/min vs $2095 \pm 25$ ct/min with Seph-BSA). DNA synthesis, evaluated after 3 and 6 days of culture, occurred only in sample no. 4 which, after 3 days of culture, showed $36\,822 \pm 300$ ct/min with Seph-PVA vs cpm $1310 \pm 10$ ct/min with Seph-BSA control.

*Proliferative responsiveness of CLL B cells to BCGF, IL-2 and INF-γ in co-stimulatory assays*
Cells no. 2, 3, 5 and 6 failing to show DNA synthesis after 3 and 6 days of culture with PMA plus A23187 or with PMA alone (Table 2), still remained unresponsive when BCGF or IL-2 were also added to the cultures. In contrast cells no. 1, 4, 7, 8 and 10, showed increased levels of DNA synthesis in response to BCGF (10% v/v) and IL-2 upon co-stimulation with PMA plus A23187 or with PMA alone (Fig. 2). Cells from patient no. 9 were not available for these assays. The responses to IL-2 were very poor in cells no. 1 and 7. A23187 did not induce DNA synthesis to BCGF or IL-2 but increased the responses induced by PMA in some cases, although in samples no. 1 and 7 the responses with PMA alone were higher than with PMA plus A23187.

**Table 2.** $^3$H-uridine and $^3$H-thymidine incorporation by CLL B cell populations cultured with PMA and A23187

| | $^3$H-uridine (ct/min × $10^{-3}$) (40 h) | | | $^3$H-thymidine (ct/min × $10^{-3}$) (3 days) | | | $^3$H-thymidine (ct/min × $10^{-3}$) (6 days) | | |
|---|---|---|---|---|---|---|---|---|---|
| CLL B cells | Medium | PMA | PMA + Iono* | Medium | PMA | PMA + Iono | Medium | PMA | PMA + Iono |
| 1 | 4·1 ± 0·30 | 14·9 ± 1·30† | 28·9 ± 2·60† | 0·9 ± 0·08 | 4·1 ± 0·40† | 4·2 ± 0·30† | 1·1 ± 0·10 | 2·2 ± 0·20 | 3·1 ± 0·30† |
| 2 | 1·0 ± 0·08 | 1·8 ± 0·17 | 3·8 ± 0·37† | 0·3 ± 0·02 | 0·4 ± 0·02 | 0·6 ± 0·03 | 0·6 ± 0·04 | 0·7 ± 0·06 | 0·8 ± 0·06 |
| 3 | 1·9 ± 0·14 | 2·8 ± 0·22 | ND | 2·5 ± 0·22 | 2·8 ± 0·26 | 1·7 ± 0·14 | 2·8 ± 0·26 | 3·0 ± 0·20 | 3·1 ± 0·28 |
| 4 | 4·9 ± 0·45 | 25·4 ± 2·43† | 107·5 ± 9·45† | 0·7 ± 0·05 | 6·6 ± 0·60† | 26·9 ± 2·55† | 0·4 ± 0·02 | 4·4 ± 0·41 | 4·5 ± 0·40† |
| 5 | 4·4 ± 0·40 | 7·2 ± 0·66 | 8·8 ± 0·75† | 0·9 ± 0·06 | 1·7 ± 0·17 | 1·5 ± 0·13 | 2·3 ± 0·21 | 2·4 ± 0·22 | 1·3 ± 0·11 |
| 6 | 3·6 ± 0·32 | 15·5 ± 1·35† | 25·8 ± 2·40† | 1·1 ± 0·10 | 1·6 ± 0·14 | 1·0 ± 0·08 | 0·7 ± 0·05 | 0·9 ± 0·07 | 1·0 ± 0·09 |
| 7 | 3·1 ± 0·30 | 29·9 ± 2·78† | 28·0 ± 2·60† | 0·1 ± 0·01 | 1·2 ± 0·11† | 1·7 ± 0·15† | 0·5 ± 0·03 | 1·7 ± 0·14† | 0·9 ± 0·76 |
| 8 | 10·1 ± 0·90 | 61·6 ± 5·50† | 59·1 ± 5·00† | 1·3 ± 0·10 | 1·9 ± 0·15 | 2·0 ± 0·18 | 1·3 ± 0·11 | 3·8 ± 0·30 | 9·5 ± 0·86† |
| 9 | 4·5 ± 0·40 | 8·8 ± 0·60 | 22·8 ± 2·02† | 0·3 ± 0·01 | 0·5 ± 0·02 | 3·4 ± 0·30† | 0·4 ± 0·03 | 0·5 ± 0·04 | 3·1 ± 0·27† |
| 10 | 5·1 ± 0·45 | 15·0 ± 1·25† | 50·0 ± 4·48† | 0·8 ± 0·07 | 15·0 ± 1·35† | 15·3 ± 1·30† | 1·1 ± 0·10 | 5·7 ± 0·50† | 12·6 ± 1·10† |

Results are expressed as mean ± s.d. of triplicate cultures.
* Iono, indicates calcium ionophore, A23187. The results in cultures with A23187 alone were not different from those obtained with medium alone.
† Indicates increased ct/min values of 100% above the medium control.
ND not done.

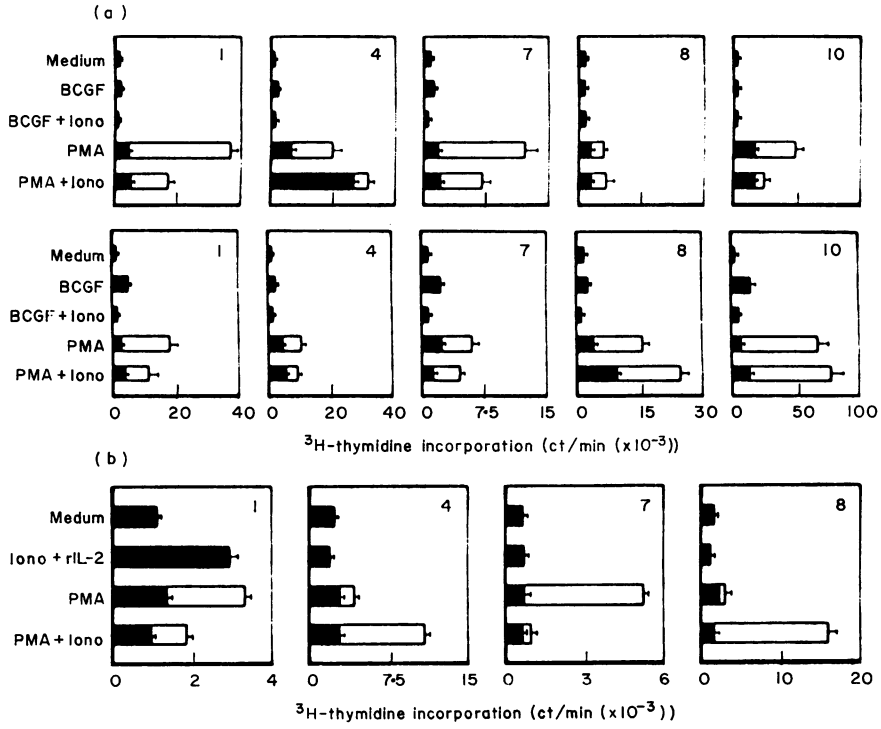


**Fig. 2.** Proliferative response of CLL B cells nos. 1, 4, 7, 8 and 10 to BCGF (a) and IL-2 (b) upon co-stimulation with PMA plus A23187 or PMA or A23187. Closed/open bars indicate results in the absence (closed) and in the presence (open) of the corresponding lymphokine. In (a) the results after 3 (upper) and 6 days (lower) of culture are shown. In (b) the results after 6 days of culture are shown. CLL B cells no. 10, not shown, exhibited consistent spontaneous proliferative response to IL-2 (see text). Cells no. 2, 3, 5 and 6 did not show detectable DNA synthesis in any of these culture conditions.

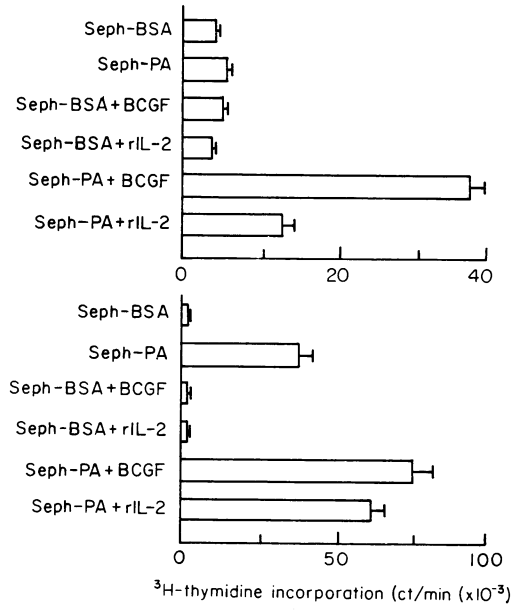


**Fig. 3.** Proliferative response of CLL B cells nos. 1 (top) and 4 (bottom) to BCGF and IL-2 in a 6-day co-stimulatory assay with Seph-PA. Seph-BSA was used as control. Cells no. 2, 3, 5, 6, 7 and 8 did not show detectable DNA synthesis in any of these culture conditions.

**Table 3.** IgM production by CLL B cells

| CLL B cells | IgM (ng/ml) | | | | |
|---|---|---|---|---|---|
| | Medium | PMA | PMA+Iono* | IL-2 | PMA+Iono+IL-2 |
| 1 | <10 | 75 | 91 | <10 | 135 |
| 2 | <10 | 215† | 295† | <10 | 382† |
| 4 | <10 | <10 | 122 | <10 | 231 |
| 6 | <10 | 60† | 210† | <10 | 360† |
| 7 | <10 | 51 | 71 | <10 | 135 |
| 8 | <10 | <10 | 25 | <10 | <10 |
| 10 | <10 | 15 | 30 | <10‡ | 120 |

Two-step cultures were done as indicated in *Materials and Methods.*

* Iono, indicates calcium ionophore A23187. IgM levels in cultures with A23187 and A23187 plus IL-2 were below 10.

† Indicates CLL B cells lacking in detectable DNA synthesis in the same assay.

‡ Indicates CLL B cells showing spontaneous proliferative response to IL-2 in the same assay.

Poor spontaneous response to BCGF after 6 days of culture was found in samples no. 1, 7 and 10 with stimulatory indexes of 4·3, 3·9 and 12·5, respectively. Spontaneous response to IL-2 was found in sample no. 10, not shown in Fig. 2. When cultured with IL-2 alone, cells from patient no. 10 showed consistent levels of DNA synthesis on day 3 (14 278 ct/min 2800 ct/min in the control) and on day 6 (20 150 ct/min vs 1215 ct/min in the control); when cultured with PMA plus A23187 and IL-2, the DNA synthesis level greatly increased on day 3, but decreased below the one obtained with IL-2 alone on day 6.

In eight samples (nos. 1–8), the capacity of Seph-PA to induce a proliferative response to BCGF and IL-2 was also examined, and compared with that of PMA plus A23187 or PMA alone. Only cells no. 1 and 4 were responsive with both co-stimulations although the magnitude of the responses was higher with Seph-PA than with PMA plus A23187 (Figs 2 and 3). CLL B cells no. 1 and 4 were those showing RNA synthesis after Seph-PA stimulation. Of note, cells no. 7 and 8 were responsive only with PMA plus A23187 stimulation, and cells no. 2, 3, 5 and 6 were unresponsive with both co-stimulations.

INF-$\gamma$, either alone or in the above co-stimulatory assays, failed to affect DNA synthesis as found by others with normal and CLL B cells (Nakagawa *et al.*, 1986; Karray *et al.*, 1987).

*IgM production by CLL B cell populations after stimulation with PMA plus A23187 in the presence or absence of IL-2 and INF-$\gamma$*

This was examined in seven CLL B cells using two-step cultures where both IgM secretion and DNA synthesis were evaluated (Table 3). A23187 alone did not promote detectable IgM secretion but increased the PMA-induced IgM levels. INF-$\gamma$ failed to affect IgM secretion (data not shown) as found by others with normal B cells (Nakagawa *et al.*, 1986). IL-2 alone did not induce IgM secretion even in sample no. 10 which showed spontaneous proliferative response to IL-2. The IgM secretion induced by PMA plus A23187 increased in response to IL-2 irrespective of the presence (nos. 1, 4, 7 and 10) or absence (nos. 2 and 6) of DNA synthesis. In cells no. 8 showing DNA synthesis, no IgM secretion was detected.

The IgM produced by each CLL B cell sample was monoclonal as indicated by ELISA determination of light chains, which were found to be of the same type as that detected on the surface of CLL B cells (data not shown).

## DISCUSSION

The $G_0$ to the $G_1$ phase transition of B cells ($G_0$–$G_1$) is characterized by cellular enlargement (i.e. blastogenesis), RNA synthesis, increase in the expression of HLA Class II antigens and acquisition of activation antigens such as CD23, 4F2, CD25 and OKT9 (Kehrl, Muraguchi & Fauci, 1984; Klaus & Hawrylowicz, 1984; Gordon & Guy, 1987). On the basis of these data this study shows that, following PMA plus A23187 stimulation, CLL B cells from different individuals are similarly able to undergo the $G_0$–$G_1$ transition and express surface activation antigens, but they are variable in the capacity to show DNA synthesis, which occurred in only some of them (six out of 10). Importantly, even in the presence of BCGF or IL-2, four out of nine CLL B cells (nos. 2, 3, 5 and 6) activated with PMA plus A23187 or PMA alone were still unable to proliferate although they were induced to express CD23, 4F2, CD25 and OKT9 antigens by PMA plus A23187 stimulation. There was no relationship between any phenotypic feature, before or after in-vitro activation, and the proliferative capability.

Activation (ie. $G_0$–$G_1$ transition) and proliferation of B cells are separately controlled events since the cell cycle of B cells is punctuated by restriction points, the major one residing in the $G_1$ phase where growth factors are needed for the progression to the S phase (reviewed in Klaus & Hawrylowicz, 1984; Gordon & Guy, 1987). The present results with PMA plus A23187 stress this point further in showing that some CLL B cells undergo cellular activation while failing to utilize exogenous BCGF and

11-2 to proliferate. Possible explanations are (a) expression of non-functional (ie. of low affinity) BCGF and IL-2 receptors; (b) a wider defective condition in other intracellular events governing the $G_1$ to the S phase progression. The former, however, seems unlikely because in cells no. 2 and 6, the IL-2 receptors induced by PMA plus A23187 were functional to permit IL-2-mediated IgM secretion without DNA synthesis. This was also found in one PMA-stimulated CLL B cell sample (Kabelitz *et al.*, 1985). Normal B cells activated *in vitro* respond to low concentrations (50 U/ml) of IL-2 with DNA synthesis with or without Ig secretion (Nakagawa *et al.*, 1986), and these effects are thought to be dependent on high-affinity IL-2 receptors. Moreover, human B cell lines exhibiting low-affinity IL-2-binding sites, are induced to secrete Ig only by high concentrations of IL-2 (1000 U/ml) (Tanaka *et al.*, 1987).

On the other hand, Ig secretion without DNA synthesis in response to PMA and PMA plus A23187, as observed in cells no. 2 and 6, has not been found in normal B cells (Aman, Gordon & Klein, 1984; Clevers *et al.*, 1985; Roifman *et al.*, 1987; unpublished results). In contrast, PMA induces IgM secretion without DNA synthesis in some CLL B cells (Larsson *et al.*, 1987). It is possible that a defective $G_1$ to S progression capability enables some CLL B cell populations to respond with Ig secretion upon stimuli that usually promote proliferation with or without Ig secretion.

Comparison of Seph-PA and PMA plus A23187 for the induction of RNA synthesis indicates that stimulation via sIg cross-linking can fail to activate most CLL B cell populations. In accord with this, CLL B cells failed to absorb BCGF activity after stimulation with anti-Ig antibodies (Perri, 1986). Data also indicate that in co-stimulatory assays with Ig ligands, the unresponsiveness to growth factors may simply reflect defective signalling via sIg cross-linking, since the proliferative responsiveness of some CLL B cells was restored when, in place of Seph-PA, PMA plus A2387 stimulation was used.

Both the defective signalling via sIg cross-linking and the proliferative unresponsiveness may be related to the cytoskeleton abnormalities known to occur in CLL B cells (Caligaris-Cappio *et al.*, 1986). CLL B cell samples with these functional abnormalities and those lacking in them are potential tools for analysing the cell structures and molecular events involved in the activation, proliferation and differentiation of B cells.

## ACKNOWLEDGMENTS

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