In vitro beta₂-microglobulin (β₂m) secretion by normal and leukaemic B-cells: effects of recombinant cytokines and evidence for a differential response to the combined stimulus of phorbol ester and calcium ionophore

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Summary. Due to the increasing therapeutic use of immunoregulatory agents and the potential effects on cellular function, we examined the modulation of in vitro beta₂-microglobulin (β₂m) production rates by ‘normal’ tonsil and leukaemic B-cells in response to a number of these agents. Tonsil B-cells responded to phorbol ester (TPA) by an increased β₂m production rate, which was further enhanced by the combined stimuli of TPA plus the calcium ionophore A23187. In marked contrast, however, lymphocytes from a majority (8/11) of B-cell malignancies showed a suppression of the TPA-induced β₂m production rate in response to the combined TPA/A23187 stimulus. These different responses of ‘normal’ and malignant B-cells were not apparent when IgM production rates were examined. The recombinant cytokines IL-1, IL-2, IFN-α, IFN-γ and TNF also enhanced β₂m production rates of both normal and leukaemic B-cells, but to a considerably lesser extent than did TPA. Bryostatin-1 increased β₂m production to a level intermediate between that obtained by TPA and the cytokines. It is suggested that β₂m production rates may correspond to the degree of B-cell differentiation, and/or to the degree of cellular ‘activation’. The results further indicate that the in vitro measurement of β₂m production provides a different index of the cellular response than that obtained by the conventional measurement of IgM production.

The 12 kDa beta₂-microglobulin (β₂m) molecule is expressed in non-covalent association with the MHC Class I (HLA-ABC) glycoprotein heavy chain (Peterson et al., 1974) on the surface of most nucleated cells (Daar et al., 1984). In addition to its release during cellular turnover, which occurs following internalisation of the HLA heavy chain (Cresswell et al., 1974), β₂m levels in the extracellular compartment may additionally result from direct cellular secretion (Nilsson et al., 1974; Conway & Poulik, 1976). In broad terms, serum β₂m concentrations have been shown to be of prognostic value in B-CLL and myelomatosis, apparently reflecting tumour cell mass (Norfolk et al., 1979; Simonsson et al., 1980; Spati et al., 1980; Child & Kushwaha, 1984; Bataille & Grenier, 1987). This is particularly striking in myelomatosis where serum β₂m can be used in the stratification and monitoring of disease. A progressive rise in serum β₂m appears to accompany the onset of active disease in B-CLL (Simonsson et al., 1980), and is paralleled by a greater in vitro β₂m production rate by the leukaemic B-cells from active disease (Simonsson et al., 1986; Totterman et al., 1986). The tumour itself is thus implicated as the source of increasing serum β₂m, but since elevated levels are also observed in inflammatory and viral conditions (Plesner, 1980), it is conceivable that these increased levels may in part be due to an immunological response to the malignancy. Various immunoregulatory agents are known to influence lymphoid cell differentiation, often with corresponding stimulation of β₂m production. Recent therapeutic advances have been noted in the treatment of conditions such as hairy cell leukaemia (HCL) by α-interferon (IFN-α) (Quesada et al., 1986; Genot et al., 1987), a lymphokine which amongst others is known to increase the expression of cell-membrane β₂m-associated determinants in vitro (Wallach et al., 1982). However, as relatively little is known about the in vivo response of leukaemic cells to these agents with respect to β₂m production, we compared the inductive capacity of eight different immunoregulatory agents on the β₂m production rates of both non-malignant tonsil and leukaemic B-cells.

Materials and methods

Source and preparation of B-cell fractions

Non-malignant human B-cells Human non-malignant B-cells were obtained from four separate tonsils which were removed during routine tonsillectomy. After teasing of the tissue with scalpel and forceps, mononuclear tonsil cells were isolated by Ficoll-Hypaque density gradient centrifugation (1.077 g ml⁻¹; Lymphoprep, Nycomed, UK). Monocytes/macrophages were depleted by adherence to plastic Petri dishes (Nunc, Gibco) for 90 min at 37°C. The lymphocyte-rich non-adherent fraction was then T-cell depleted using a standard sheep erythrocyte rosetting technique whereby non-rosetting B-cells were obtained from the interface of Ficoll and sheep erythrocytes. The resulting tonsil lymphoid population comprised 0–1% monocytes (CD14⁺), 1–2% T-cells (CD2+, CD5⁺), and exceeded 90% B-cells as defined by expression of CD19, CD20 and CD21.

Leukaemic B-cells Leukaemic cells from a total of 11 cases (clinical and haematological data detailed in Table I) were examined in this study. These were diagnosed as chronic lymphocytic leukaemia (B-CLL; n = 3), ‘prolymphocytoid variants’ of B-CLL (CLL-Pro; n = 5), non-Hodgkin’s lymphoma (B-NHL; n = 1), prolymphocytic leukaemia (B-PLL; n = 1) and hairy cell leukaemia—proliferative variant (HCL-v; n = 1) on the basis of morphological appearances and immunophenotypic profiles as previously described (Melo et al., 1986; Drexler & Scott, 1989). Mononuclear leukaemic cells were purified by density gradient centrifugation of heparinised blood on Ficoll-Hypaque and the harvested cells were examined immediately for the expression of lymphoid-associated membrane determinants by indirect immunofluorescence in suspension using a microtitre plate technique (Campana & Janossy, 1986). Immunophenotyping reagents included polyclonal goat anti-human immunoglobulin heavy- and light-chain antibodies, and a wide range of murine anti-human monoclonal antibodies (Table II).

Culture conditions

Fractionated tonsil and leukaemic B-cells were cultured for up to 10 days in RPMI 1640 medium (Gibco) supplemented...
Recombinant (r) cytokines used in this study included: rTNF (tumour necrosis factor; Knoll AG, BASF, Ludwigshafen, FR Germany), rIFN-γ, IL-1 and IL-2 (γ-interferon and interleukins 1 and 2; BioGen, Geneva, Switzerland) and rIFN-α (Kirby, Warwick, UK). The specific activity of rTNF was 6.63 × 10^6 U mg⁻¹ protein, of IFN-α 2.5 × 10^6 U mg⁻¹ protein, of IFN-γ 3.3 × 10^6 U mg⁻¹ protein, of IL-1 5 × 10^6 U mg⁻¹ protein, and of IL-2 1.7 × 10^6 U mg⁻¹ protein. These cytokines were stored frozen and diluted prior to use in RPMI 1640 medium. Other inducers examined included: bryostatin-1, which was extracted from the marine bryozoa Bugula neritina; TPA (12-O-tetradecanoylphorbol 13-acetate; Sigma); and the calcium ionophore A23187 (Sigma). Bryostatin-1, TPA and A23187 were dissolved in DMSO at 10⁻³ M, stored frozen and then further diluted to final concentrations in culture medium.

### Table II Immunological reagents used in this study for characterisation of normal tonsil and leukaemic B-cells

| Cluster designation | Principal reactivity | Antibody | Source |
|---------------------|----------------------|----------|--------|
| **Monoclonal antibodies** | | | |
| CD2 | T-cells | RFT-11 | RFH |
| CD5 | T-cells (B-PLL) | RFT-1 | RFH |
| CD6 | T-cells (B-PLL) | RFT-12 | RFH |
| CD10 | Pre-B cells (CALLA) | RPAI-13 | RFH |
| CD14 | Monocytes | VIM-13 | Dr Knapp |
| CD15 | Monomoyeloid cells | VIM-D5 | Dr Knapp |
| CD19 | B-cells | RFB-9 | RFH |
| CD20 | B-cells | RFB-7 | RFH |
| CD21 | B-cells | RFB-6 | RFH |
| CD22 | B-cells | RFB-4 | RFH |
| CD25 | IL-2 receptor | TAC | Dr Waldmann |
| CD38 | Plasma cells | OKT-10 | Ortho |
| HLA-DR | B-cells/monocytes | RFDR-2 | RFH |
| – ‘Late’ B-cells | FM2C | Dr Zola |
| – ‘Activated’ B-cells | UCHB1 | Dr Armitage |
| – ‘Activated’ B-cells | B7 | Dr Freeman |
| – ‘Activated’ B-cells | B7 | Dr Freeman |
| **Polyclonal antibodies** | | | |
| anti-kappa | SBA | |
| anti-lambda | SBA | |
| anti-lgM | SBA | |

*Cluster designations as defined by the four International Workshops. *Antibody sources: RFH, Royal Free Hospital, London; Ortho, Ortho Diagnostics; SBA, Southern Biotechnology Assoc, Birmingham, AL, USA; Dr Knapp, Vienna, Austria; Dr Waldmann, Bethesda, MD, USA; Dr Zola, Bedford Park, Australia; Dr Armitage, London, UK; Dr Freeman, Boston, MA, USA.*

### Analysis of beta-2-microglobulin (ß2m) secretion

*In vitro ß2m production was determined by radioimmunoassay of cell-culture supernate samples, taken on successive days from cultures maintained at 2 × 10⁶ cells ml⁻¹ for up to 10 days. Mean rates of synthesis (ng ml⁻¹ (10⁶ cells⁻¹ day⁻¹) were calculated over the observed period of linear production, normally between days 2 and 6. Supernate ß2m concentrations were measured using a modification of a previously described method (Swanson et al., 1982). Briefly, monoclonal anti-ß2m antibody L368 (American Type Culture Collection), which was purified from hybridoma culture medium by Protein-A chromatography, was incubated with 100 µl of 1:10 tonsil or leukaemic B-cell culture supernate and a standard amount of ß2m-labelled soluble ß2m. Following overnight incubation at 4°C and precipitation of immune complexes with 18% polyethylene glycol (PEG), the amount of precipitated activity was measured and the culture supernate ß2m concentration thus calculated. Three internal ß2m standard preparations, representing low (4.3 ng ml⁻¹), mid-range (37.5 ng ml⁻¹) and high (99.9 ng ml⁻¹) ligand concentrations, gave inter-assay variation coefficients (CV) of 16.3%, 6.7% and 6.5% respectively (n = 23). Intra-assay reproducibility, determined by replicate measurement of a single sample (mean 30.7 ng ml⁻¹; n = 10), was 4.5%.*

### Analysis of immunoglobulin production

Immunoglobulin production by cultured B-cells was measured using a specific ELISA as described in detail previously (Drexler et al., 1988).

### Results

ß2m production rates were determined in three cases of B-CLL, five cases of CALL-Pro, one case each of B-NHL, B-PLL and HCL and four (T-cell depleted) tonsil B-cell fractions cultured with one of eight mitogenic or immunoregulatory agents, or combination thereof. Pheno-
Dose-response of normal and malignant B-cells to TPA

The effect of serial concentrations of the phorbol ester TPA can be seen from Figure 1. TPA in concentrations below \(10^{-9}\) M were largely ineffective in stimulating \(\beta_m\) production above that of the control value, for both tonsil \((n = 2)\) and leukaemic \((n = 4)\) B-cells. An apparent difference in the threshold of response to TPA can be observed between tonsil and leukaemic B-cells, since three of the four cases of B-CLL examined showed an increase in \(\beta_m\) secretion, compared to spontaneous production, at a TPA concentration of \(10^{-8}\) M. In contrast, both of the B-cell fractions studied only showed a clear response to TPA at \(10^{-8}\) M, suggesting that B-CLL cells may be slightly more sensitive to TPA than tonsil B-cells.

\(\beta_m\) production by normal and malignant B-cells: effects of TPA, the calcium ionophore A23187 and bryostatin-1

The \(\beta_m\) production rates of tonsil and leukaemic B-cells cultured with TPA, A23187 and bryostatin-1 either alone or in combination are shown in Table IV.

![Figure 1. Dose–response curve of \(\beta_m\) production in the presence of increasing TPA concentrations (\(10^{-11}\) to \(10^{-8}\) M). Results are shown as the mean \(\beta_m\) production rates (ng (10^6 cells)^{-1} ml^{-1} day^{-1}) for two normal tonsil B-cell fractions (open circles: curves a and b) and four malignant B-CLL-Pro B-cell fractions (filled circles: curves c–f). C indicates the spontaneous (control) \(\beta_m\) production obtained in the absence of TPA.](image-url)

Tonsil B-cells

The mean unstimulated (control or spontaneous) \(\beta_m\) production by tonsil B-cells \((n = 4)\) was 13.4 (range 10.8–14.6) ng ml^{-1} (10^6 cells)^{-1} day^{-1}, while that of TPA-treated tonsil B-cells was 50.9 (range 41.3–69.4). The \(\beta_m\) production rate in the presence of TPA alone was, however, less than that obtained when TPA was supplemented with the calcium ionophore A23187; this combination increased additively the mean \(\beta_m\) production rate to 71.2 (range 62.7–78.4) ng ml^{-1} (10^6 cells)^{-1} day^{-1}.

Malignant B-cells

The mean unstimulated \(\beta_m\) production by leukaemic B-CLL/CLL-Pro cells \((n = 8)\) was 14.3 (range 6.9–23.1) ng ml^{-1} (10^6 cells)^{-1} day^{-1}, with a considerably higher spontaneous level of secretion observed for the non-CLL (B-NHL, B-PLL and HCL-\(\nu\)) cases (mean 30.9, range

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**Table III** Immunophenotypic characteristics of enriched normal tonsil B-cells and leukaemic B-cells examined in this study*

| Surface Ig | B-CLL | CLL-Pro | B-NHL | B-PLL | HCL-\(\nu\) |
|------------|-------|---------|--------|--------|-------------|
| **I**      |       |         |        |        |             |
| kappa      | n.t.  | n.t.    | n.t.   |        |             |
| lambda     | n.t.  | n.t.    | n.t.   |        |             |
| IgM        | n.t.  | n.t.    | n.t.   |        |             |
| **II**     |       |         |        |        |             |
| CD5        | <5    | <5      | <5     | <5     | <5          |
| CD6        | n.t.  | n.t.    | n.t.   | <5     | <5          |
| CD10       | n.t.  | n.t.    | n.t.   | <5     | <5          |
| CD19       | 82    | 57      | 88     | 96     | 96          |
| CD20       | 74    | 67      | 86     | 94     | 98          |
| CD21       | 61    | 47      | 73     | 85     | 91          |
| CD22       | n.t.  | n.t.    | n.t.   | <5     | <5          |
| HLA-DR     | n.t.  | n.t.    | n.t.   | <5     | <5          |
| **III**    |       |         |        |        |             |
| FMC7       | n.t.  | n.t.    |        | 85     | 22          |
| UCHB1      | n.t.  | n.t.    |        | <5     | <5          |
| B5         | n.t.  | n.t.    |        | 98     | 90          |
| B7         | n.t.  | n.t.    |        | <5     | <5          |
| CD25       | n.t.  | n.t.    |        | <5     | <5          |
| CD38       | n.t.  | n.t.    |        | <5     | <5          |
| **IV**     |       |         |        |        |             |
| T and myeloid markers | | | | | |
| CD2       | <5    | <5      | <5     | <5     | <5          |
| CD14      | <5    | <5      | <5     | <5     | <5          |
| CD15      | <5    | <5      | <5     | <5     | <5          |

*Results are shown as the percentages positive cells expressing any given determinant. Case groups: B-CLL, chronic lymphocytic leukaemia; CLL-Pro, prolymphocytoid variant of B-CLL; B-NHL, non-Hodgkins lymphoma; B-PLL; prolymphocytic leukaemia; HCL-\(\nu\), proliferative variant of hairy cell leukaemia.
25.8–37.8). TPA treatment induced mean βm production rates for B-CLL/CLL-Pro cells of 56.7 (range 29.7–84.9) ng ml⁻¹ (10⁶ cells)⁻¹ day⁻¹, compared to 105.3 (range 76.5–151.0) for non-CLL cases. However, in contrast to tonsil B-cells, the βm production rates obtained with TPA in combination with A23187 were lower in 8/11 cases of B-cell malignancy than that observed for TPA alone; with mean production rates for TPA plus A23187 being 47.4 (range 23.1–81.2) and 92.9 (range 66.6–141.9) ng ml⁻¹ (10⁶ cells)⁻¹ day⁻¹ for B-CLL/CLL-Pro and non-CLL cases respectively.

Thus the response of tonsil and leukemic B-cells to the combined stimuli of TPA and A23187 differed. TPA-induced βm production was enhanced by A23187 in 4/4 tonsil B-cells (mean increase relative to control from 3.8 to 5.3), but was suppressed in 8/11 cases of B-cell malignancies examined (mean decrease relative to control from 3.3 to 3.4 to 3.0 for B-CLL and non-CLL cells respectively), as illustrated in Figure 2.

| Normal tonsils | Control* | TPA* | Bryo-I* | A23187* | TPA/A23187* | Bryo-I/A23187* |
|---------------|----------|------|--------|---------|-------------|----------------|
| I             | 14.1     | 44.4 (3.1)* | n.t.   | 19.7 (1.4) | 69.3 (4.9) | 43.7 (3.1) |
| II            | 14.6     | 69.4 (4.7)* | 35.0 (2.4) | 27.7 (1.9) | 78.4 (5.4) | 73.0 (5.0) |
| III           | 14.2     | 41.3 (2.9)* | 35.7 (2.5) | 27.0 (1.9) | 62.7 (2.9) | 44.0 (3.1) |
| IV            | 10.8     | 48.7 (4.5)* | 27.0 (2.5) | 20.5 (1.9) | 74.4 (6.9) | 76.7 (7.1) |

*Results are shown as absolute βm production rates (ng ml⁻¹ (10⁶ cells)⁻¹ day⁻¹) in the presence or absence (control) of inducers. Production rate ratios, relative to the control (spontaneous) value, are shown in parentheses. **TPA concentrations used were either 10⁻⁸ or 2 x 10⁻⁹ M respectively.

Bryostatin-1 was a less potent inducer of βm production than TPA, but when used in conjunction with A23187, an increased βm production rate was observed for both tonsil and leukemic B-cells.

Correlation between βm production and IgM secretion
This study also examined possible correlations between βm and IgM secretion rates in nine cases of B-cell malignancy. The mean spontaneous IgM production rates, determined over a period of 5 days and expressed as μg ml⁻¹ (10⁶ cells)⁻¹ day⁻¹, for B-CLL (n = 3), CLL-Pro (n = 4) and B-NHL/B-PLL (n = 2) cases were <0.01, 0.07 and 0.11 μg ml⁻¹ (10⁶ cells)⁻¹ day⁻¹ respectively, suggesting a common relationship between the levels of βm and IgM production with the stage of leukemic B-cell differentiation (r=0.88; P<0.05). However, in contrast to the broadly consistent 3.5-fold increase in βm production induced by TPA, the rate of IgM production increased by an approximate factor of 25 for the B-CLL cases, a mean factor of 14 for CLL-Pro cases and only 5 for the B-NHL/B-PLL cases. Furthermore, when TPA was supplemented with A23187, the rates of IgM production for all cases examined were generally similar to that observed with TPA alone, and in contrast to the suppressive effect of A23187 on βm production in TPA cultures.

βm production by normal and malignant B-cells; effects of rIFN-γ, rIFN-α, rIL-1, rIL-2 and rTNF (Table V)

Of the five different cytokines studied, IFN-γ induced a greater increase in βm production than did IFN-α, with relative production rates compared to control levels of 1.9 (range 1.5–3.2; n = 11) and 1.5 (range 1.3–1.8; n = 6) respectively. Similarly, IL-2 induced βm production to a greater degree than IL-1, with factors of 1.7 (range 1.0–2.3; n = 10) and 1.2 (range 0.9–1.7; n = 6) respectively. Culture in the presence of TNF alone did not significantly increase βm production (1.1; range 0.9–1.2; n = 11) although TNF and IFN-γ in combination produced a value of 1.8 (range 1.5–2.0; n = 7). There were no apparent differences in the response to these cytokines between non-malignant tonsil and leukemic B-cell fractions.

Discussion
This study has examined the effect of various protein kinase C activators and recombinant cytokines on the in vitro rate
of $\beta_m$ production by tonsil and leukaemic B-cells. The spontaneous (control) $\beta_m$ production rates for the tonsil B-cell fractions were broadly similar to those observed for the 'early' B-cell malignancies (B-CLL and CLL-Pro) whereas considerably higher unstimulated $\beta_m$ production rates were found in all three 'late' B-cell proliferations (B-NHL, B-PLL and HCL-v). This appears contradictory as tonsil B-cells, despite their heterogeneity, show a more mature immunological membrane phenotype than B-CLL/CLL-Pro cells (Jones et al., 1989) and, in terms of differentiation, are closer in ontology to malignant B-cell expansions such as B-NHL, B-PLL and HCL-v.

Of the activators and cytokines examined, TPA was the most potent stimulator of $\beta_m$ production in both tonsil and leukaemic B-cells. Compared to the control cultures, TPA increased $\beta_m$ production in the 11 non-malignant and leukaemic cases studied by a mean factor of 3.5. In addition, with the exception of one CLL-Pro case (PR) which showed a marked increase in TPA-induced $\beta_m$ production and was the only B-cell malignancy examined with evidence of a proliferative component (1% nuclear Ki-67+), there were no significant differences in the levels of increased production between tonsil and leukaemic B-cells. However, different responses were apparent for tonsil and leukaemic B-cells when TPA was used in combination with the calcium mobilising ionophore A23187. An enhancement of TPA-induced $\beta_m$ production occurred in tonsil B-cells, whereas A23187 appeared to exert a suppressive effect on TPA-induced production in most of the B-cell malignancies. When used singly, A23187 failed to enhance $\beta_m$ production much above control levels in three of four cases of B-cell malignancy, but induced a modest increase in production rate for all four tonsil B-cell cultures. Although we are not aware of the significance of these different responses between 'normal' and leukaemic B-cells, a potential consideration is that the former become activated and proliferate in the presence of TPA whereas the latter are induced to differentiate and undergo apoptosis (Bartoglio, 1983; Drexler et al., 1988). However, the one case included in this study with evidence of 'low level' proliferation (case PR) showed response patterns to the stimulus of A23187 and TPA/A23187 which were more similar to the other (non-proliferating) leukaemic B-cell cases than to tonsil B-cells. The ratio of TPA-induced $\beta_m$ production to spontaneous $\beta_m$ production was particularly striking in this case, but whether this finding is a reflection of the proliferation process is not known.

A previous study of leukaemic B-cells in 22 cases of B-CLL (Simonsson et al., 1986) showed that the mean spontaneous in vitro $\beta_m$ production rate in 12 patients with progressive disease (NCI committee criteria for CLL) was 0.07 (s.d. ± 0.05) ng ml⁻¹ from 5 x 10⁶ cells in 24 h, compared with 0.02 (0.01) ng ml⁻¹ for 10 patients with non-progressive disease. These values correspond to 140 (s.d. ± 100) and 40 (s.d. ± 20) ng ml⁻¹ (10⁶ cells⁻¹) day⁻¹ respectively. In a separate study by the same group (Totterman et al., 1986), the TPA-treated (1.6 x 10⁻⁸ M) B-CLL cells from 15 cases of 'active disease' produced the equivalent of 160 (s.d. ± 60) ng ml⁻¹ (10⁶ cells⁻¹) day⁻¹, compared with a control rate of 73.3 (s.d. ± 30) ng ml⁻¹ (10⁶ cells⁻¹) day⁻¹, whereas 17 cases of 'inactive disease' produced 43.3 (s.d. ± 30) and 26.7 (s.d. ± 20) ng ml⁻¹ (10⁶ cells⁻¹) day⁻¹, respectively. Despite methodological differences between the two studies, these values are in general agreement with our own, although their study did not distinguish between CLL and the clinically more aggressive PLL-Pro variant.

No apparent differences in the response of tonsil and leukaemic B-cells to the immunoregulatory agents IL-1, IL-2, IFN-γ, IFN-α and TNF were seen. The stimulatory capacity of IL-2 was found to be greater than IL-1, and IFN-γ was greater than IFN-α but in almost no case was the stimulation of $\beta_m$ production as extensive as that produced by TPA or bryostatin-1. Although TNF and IFN-γ have been reported to act synergistically in enhancing MHC class I (and therefore $\beta_m$) expression (Pfizenmaier et al., 1987), this was clearly not the case in terms of $\beta_m$ export, as the production rate by IFN-γ/TNF-stimulated cells was not apparently greater than that of cells stimulated by IFN-γ alone.

The precise significance of enhanced $\beta_m$ production with stimulation has not been established, but it may simply reflect the degree of differentiation thus induced. It is generally accepted that the stage of B-cell differentiation is broadly reflected by the IgM secretion rate. However, it is apparent from this study that, despite the slight ($P < 0.05$) correlation between the spontaneous $\beta_m$ and IgM secretion rates, that the degree of leukaemic cell differentiation appears more closely correlated with the $\beta_m$ production rate. Moreover, the suppression in TPA-induced $\beta_m$ production rates observed when TPA and A23187 were used in combination was not apparent with IgM secretion rates. The measurement of $\beta_m$ production rates could therefore be of potentially more value than IgM production rates in assessing the response of leukaemic cells to biomodulating agents. Hence, using $\beta_m$ production rate as an index of response, the recombinant cytokines IL-1, IL-2, IFN-γ, IFN-α and TNF were considerably less potent than TPA (with or without calcium ionophore), which is at least consistent with previous observations (Drexler et al., 1988; Drexler, unpublished observations) showing that such stimuli are not at all, or only weakly, efficient in inducing B-CLL differentiation.

Alternatively, the level of spontaneous $\beta_m$ production might represent a marker for the degree of activation of a B-cell. It is noteworthy that in 8/11 of the leukaemic populations examined, the control $\beta_m$ production rates were higher than those of the tonsil B-cell fractions. Based on their
cytological and surface antigen (Slg) features, B-CLL cells were originally considered as the malignant counterparts of small resting B-cells (Robert et al., 1986). However, recent functional studies suggested that B-CLL cells are arrested in an 'activated' state (Beiske et al., 1988) that is reflected by the expression of one or more activation-associated antigens such as FMC7, UCHB1, CD25, B5 or B7 (Table III). Furthermore, the morphology of B-PLL cells and B-CLL cells in 'prolymphocytoid transformation' (CLL-Pro) resembles that described for activated normal B-cells (Melo et al., 1986). Thus, viewed in these terms, our data on spontaneous Bm production supports the contention that the leukaemic cells from B-CLL, CLL-Pro and, in particular, B-PLL represent cells at an activated stage of B-cell differentiation.

We also analysed the effects of the new protein kinase C stimulator bryostatin-1. In contrast to phorbol esters, bryostatin-1 lacks tumour-promoting activity and even has anti-neoplastic properties (Blumberg, 1988). The naturally occurring bryostatin-1, a macrocyclic lactone isolated from the marine bryozoan Bugula neritina, binds to protein kinase C and, like TPA, stimulates its activity. However, bryostatin-1 induces only some of the responses obtained with phorbol esters and may even block TPA-mediated responses in some experimental situations (Drexler et al., 1989). The 'weaker' effects of bryostatin-1 on some parameters might be explained by a more transient action for bryostatins associated with accelerated breakdown of protein kinase C. In our study, bryostatin-1 was effective in inducing Bm production, particularly in combination with A23187, but the levels of Bm induced by bryostatin-1 used singly were significantly lower than those found in cultures exposed to TPA. In tonsil B-cells, the combination of bryostatin-1 and A23187 was not as stimulatory for Bm production as was the TPA/A23187 combination, whereas for leukaemic B-cells, the two combinations had approximately equal potency. This was probably an indirect result, in these particular cases, of the suppressive effect on TPA-induced Bm production by A23187.

Finally, this study supports previous evidence that the signal transduction pathway distal to protein kinase C is intact in B-CLL and related leukaemic cells and can be stimulated effectively by protein kinase C agonists such as TPA or bryostatin-1 (Drexler et al., 1988, 1989).

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