Analysis of Signal Transduction in B Chronic Lymphocytic Leukemia Cells

By Hans G. Drexler, Malcolm K. Brenner, Elaine Coustan-Smith, Suzanne M. Gignac, and A. Victor Hoffbrand

We report here experiments on the analysis of cellular signal transduction in a series of patients with chronic B cell disorders (B cell chronic lymphocytic leukemia [B-CLL] and prolymphocytic leukemia). We compared the response of the leukemic cells with primary external signals (interleukin 2 [IL-2] or B cell differentiation factors [BCDF or IL-6]) with their response to secondary inducers (the phorbol ester 12-0-tetradecanoylphorbol-13-acetate [TPA] or the calcium ionophore A23187) that circumvent the first part of the signal transduction pathway by directly activating the key enzyme protein kinase C. One BCDF was synthesized by mitogen-activated peripheral blood B lymphocytes; a second BCDF was constitutively produced by the human bladder carcinoma cell line T24. Changes in morphology, Tac (IL-2 receptor) expression, RNA synthesis measured by [3H]-uridine uptake, and immunoglobulin production tested by enzyme-linked immunosorbent assay were used as parameters of successful signal transduction.

TPA alone and TPA plus A23187 (synergistically) effect

ively initiated differentiation in all the leukemia cases. Neither IL-2 nor BCDF (singly or in combinations) caused equivalent responses. On the other hand, IL-2 and BCDF produced a substantial differentiation effect on normal B lymphocytes. Our data suggest that (a) B-CLL cells are able to respond to direct stimulation of the second messenger pathway (through protein kinase C) but not to the physiological stimuli IL-2 or BCDF; (b) the defect in signal transduction appears to be located upstream of protein kinase C (a possible candidate is a G protein); and (c) malignant B cells may spontaneously or after treatment with inducers express the IL-2 receptor (Tac antigen) in the absence of a functional differentiating response to IL-2; and (d) signs of proliferation/differentiation in B-CLL samples after incubation with IL-2 or BCDF might be due to contamination of the cell populations with residual normal B cells.

HUMAN B chronic lymphocytic leukemia (B-CLL) cells are known to respond to the phorbol ester 12-0-tetradecanoylphorbol-13-acetate (TPA) by acquiring distinct morphological and cytochemical changes, producing immunoglobulin, and newly expressing a panel of differentiation-associated surface markers.1,3 Phorbol esters mimic physiological signals by activating their intracellular receptor protein kinase C (PKC).4 This phospholipid- and Ca2+-dependent serine/threonine protein kinase plays a key role in numerous cellular responses including regulation of cell growth, differentiation, and cellular metabolism by de novo activation of genes, mRNA transcription, and synthesis of new proteins.5,6 Critical components in this system are phosphatidylinositol-4,5-bisphosphate (PI(2,4,5)) and calcium. Interaction of a ligand with its cell surface receptor stimulates a phosphodiesterase (phospholipase C) to hydrolyze PI(2,4,5). The products of this degradation are diacylglycerol (DG) and inositol-1,4,5-trisphosphate (IP3). These two second messengers trigger two synergistically acting signal pathways. DG activates PKC, which is translocated from the cytosol to the cell membrane. This translocation appears to be an important event in signal transduction. Upon activation PKC catalyzes the phosphorylation of numerous proteins by using adenosine triphosphate as a phosphate donor.5,9

On the other arm of the two parallel pathways, the second product of PI(3,4,5)P3 degradation, IP3, causes the increase in intracellular Ca2+. Presumably, a rapid transient rise in Ca2+ is due to a massive release from intracellular stores (plasma membrane, endoplasmic reticulum), whereas a sustained increase is caused by Ca2+ influx through the activation of transmembrane Ca2+-permeable channels by IP3 and/or IP4 (which is derived by phosphorylation of IP3).10,11 A rise in the cytosolic Ca2+ concentration activates Ca2+-dependent protein kinases, namely, PKC and a calmodulin-dependent protein kinase.

Experimentally, these second-messenger pathways (PKC activation and Ca2+ mobilization) can be dissociated and investigated separately by using chemicals that directly activate one of them. DG interacts with PKC at the same site as TPA and by a similar mechanism.3 Phorbol esters are more potent and less rapidly metabolized (inhibiting the action of DG lipase).5,12 On the other hand, calcium ionophores (such as A23187 or ionomycin) can imitate the physiological signal of IP3 and increase the intracellular Ca2+ concentration.6,13 A23187 is a lipid-soluble compound that raises the intracellular Ca2+ concentration by electroneutral exchange of Ca2+ and H+ across the cell membrane.10 One suggested mechanism for the synergism between phorbol esters and calcium ionophores is that the rise in cytosol Ca2+ level that is produced by A23187 promotes within seconds translocation and binding of PKC to the cell membrane; thereby it increases the availability of phospholipid cofactor and the affinity of binding of PKC for phorbol ester14 and thus "primes" the system for activation by phorbol ester.15 In other words, calcium ionophore converts the receptor for phorbol ester from a low- to a high-affinity state.16

A synergy between TPA and A23187 was seen with respect to morphological and functional changes in normal cells17-20 and in B-CLL cells.21 Because these direct activators of PKC circumvent the natural receptor-mediated hydrolysis

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of PIP₂, these results show that the signal transduction system downstream of PKC functions in B-CLL cells.

In the present investigation we attempted to answer whether in B-CLL cells the early part of the signal transduction pathway can respond appropriately to external signals such as B cell differentiation factor (BCDF) or interleukin-2 (IL-2). Recently, the designation IL-6 has been proposed for BCDF. As parameters of the end points of the activation events we chose incorporation of 3H-uridine to indicate RNA synthesis and production of monoclonal immunoglobulin (lg).

MATERIALS AND METHODS

Patients and normal controls. Seventeen patients with chronic B cell disorders were selected from a routine hematology clinic. Emphasis was put on choosing patients with WBC counts of at least 30 x 10⁹/L. Each patient was tested on at least two occasions. Fourteen patients had CLL; the remaining three cases were prolymphocytic leukemia (PLL) or CLL in prolymphocytoid transformation (CLL/PL, n = 1) as judged morphologically by the number of prolymphocytes present. Diagnosis was established by morphology, clinical data, and immunologic studies. Clinical data at the time of sampling are presented in Table 1. Surface marker analysis identified all cases as the B cell (CD19+). Venous blood was also obtained from three healthy adult volunteers.

Cell preparations. Mononuclear cells were isolated from normal or leukemic heparinized peripheral blood by Ficol-Hypaque gradient centrifugation (Lymphoprep, Nyegaard, Oslo). Monocytes were removed from all samples by incubation on plastic dishes with 2,000 rad Escherichia coli (vol/vol) BCDF; and 10% (vol/vol) T24 supernatant. After onset of culture period no medium was added. At 24-hour intervals from days 1 to 6, cells were harvested for analysis of morphology, RNA synthesis and Ig production.

Flow cytometric analysis of IL-2 receptor expression. Day 0 cells and cells cultured in the presence or absence of stimulants for three days were stained with the Tac MoAb (anti-IL-2 receptor antibody, CD25); this was followed by reaction with FITC-conjugated, isotype-specific F(ab')₂, reagents as described earlier. Analysis was performed on an EPICS V cell sorter (Coulter Electronics, Hialeah, FL) using an argon laser exciting at 488 nm. Residual dead cells or cell aggregates were gated out according to forward-angle and 90° light scattering. At least 10,000 viable cells were counted; the results were plotted by the EASY 88 computer program (Coulter).

Preparation of BCDF. The method used has been described previously. T cells irradiated with 2,000 rad were cultured with non-T cells at a ratio of 4:1 at a concentration of 2 x 10⁶/mL in RPMI 1640 medium supplemented with 10% FCS. At the beginning of culture 5 μg/mL of pokeweed mitogen (Sigma) was added. After 48 hours, the cells were washed with fresh Iscove’s medium and

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<th>Table 1. Clinical Data of Patients Studied</th>
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Abbreviations: C, cyclophosphamide; H, hydrocortisone; O, Oncovin; P, prednisolone.

After depletion of T cells and monocytes, 90% or more of the cells in the leukemic samples were CD20+ (RFB-7) and surface immunoglobulin-positive with less than 1% T cells (CD3+, Leu-4+) and less than 1% monocytes (CD14+, Leu-M3+). In the normal samples the sheep erythrocyte-negative cell populations (nominal B cell populations) contained 40% to 50% CD20+ B cells, about 5% CD3+ T cells, and 1% to 2% CD14+ monocytes.

Routine immunophenotyping. The surface marker profiles of the samples were determined by using the routine leukemia phenotyping program as performed at the Royal Free Hospital (Department of Immunology). The cells were stained with direct or indirect immunofluorescence in suspension according to the microtitrater plate methodology and analyzed under an epi immunofluorescence microscope equipped with appropriate filters.

Briefly, the cells were first incubated with specific murine monoclonal antibodies (MoAbs, Table 2) and then stained with fluorescein isothiocyanate (FITC) or tetramethyl rhodamine isothiocyanate (TRITC)-conjugated goat antimouse reagents. Surface immunoglobulin expression was tested with directly FITC or TRITC conjugated, isotype-specific, and light chain-specific goat anti-human immunoglobulin antiserum. The results of the phenotyping are shown in Table 2.
recollected with modified Iscove's medium. Twenty-four hours later, the supernatants were harvested and filtered. This medium containing BCDF activity was added to the experimental culture cells at a 10% final volume.

**Bladder carcinoma cell line T24 supernatant.** The human bladder carcinoma cell line T24,28 constitutively secretes a molecule with BCDF-like activity. Recently, the molecular cloning and structural analysis of the complementary DNA (cDNA) encoding human BCDF (IL-6) from a human T cell line (TCL-Nal) that constitutively produced large amounts of BCDF/BSF-2 has been reported.30 Comparative analysis of T24 cells and TCL-Nal cells showed that the same mRNA was transcribed in both cell lines.31 Other authors suggested that the cDNA cloned from TCL-Nal cells is identical to interferon-β.32,33

Filtered supernatants from T24 cell line cultures were a generous gift from Dr John Shields (Institute of Child Health, London). T24 supernatant was added to the culture plates at a final concentration of 1% (vol/vol) which had been shown to be optimal in preliminary experiments. We refer to this supernatant as T24 (-BCDF). T24 supernatant was free of IL-1, IL-2, interferon-α, and interferon-γ activity.34

**Morphological studies.** The morphology of cells after cultivation (adherence and formation of clusters) was observed in situ on the cells in the original culture wells under an inverted phase-contrast microscope. Aliquots were also taken to prepare cytosins in a cytocentrifuge (Shandon-Elliot Cytospin, Sewickley, PA). The cytosin slide preparations were stained with standard May-Grünwald-Giemsa and were examined by light microscopy.

**Cell size measurements.** Sizes of cells fixed in 8% formalin were measured by using the forward-angle and 90° light scattering parameters of the flow cytometer. Channel numbers on an arbitrary linear scale representing the mean peaks of each curve were compared.

**RNA synthesis determination.** Cells were pulsed with 1 μCi [5,6-3H]-uridine (specific activity, 37 Bequerel (MBq)/mCi; Amersham International, Amersham, UK) for one hour. Cells were harvested with a Multimash 2000 (Dynatech Laboratories, Inc., Bvinghurst, UK), and incorporation of 3H-uridine into RNA was determined by standard liquid scintillation β counting (LKB 1210 UtrroBeta, LKB Instruments, Bromma, Sweden).

**Quantification of Ig production by ELISA.** Ninety-six-well Dynatech ELISA plates were coated overnight with class-specific rabbit antihuman IgM (Dako Laboratories, Weybridge, UK) or goat antihuman IgG (Sigma). The respective intermediate washing steps were done with three cycles of phosphate-buffered saline containing 0.02% Tween 20. Unoccupied binding sites (to prevent non-specific binding) were saturated by incubation with a blocking buffer composed of 0.2% bovine serum albumin and 0.2% gelatin dissolved in 0.1% sodium azide solution for two hours at 37°C. Standard human IgM and IgG were prepared by doubling dilutions in blocking buffer. The various standard dilutions and aliquots of the unknown Ig-containing supernatants and several dilutions thereof were transferred to the test plates and were incubated for two hours. After washing, appropriate dilutions of alkaline phosphatase-conjugated goat antihuman antibodies specific for IgM or IgG (Sigma) were added for two hours. P-nitrophenyl phosphate (Sigma) was used as a substrate. After 15 to 45 minutes, the released p-nitrophenolate was measured at 405 nm on a multispan ELISA reader (Skatron). Absorbance curves for samples and standards were plotted, and Ig concentrations were calculated by using the Rubycrite ELISA program (London). All samples were tested for IgM and IgG. For the determination of monoclonality the production of κ or λ light chains were examined by using goat antihuman κ or λ antibodies conjugated to alkaline phosphatase as a second layer.

**Cell cycle analysis using Ki-67.** Cytospin slide preparations were fixed with cold methanol and stained for 30 minutes with MoAb Ki-67 (Dako); this was followed by 30 minutes of incubation with FITC-conjugated goat antiume Ig antibody and analyzed by immunofluorescence microscopy. The Ki-67 nuclear antigen is expressed on all proliferating cells, preferentially during the late G1, S, M, and G2 phases of the cell cycle whereas cells in the G0 phase consistently lack the antigen.35

**Results**

Morphological changes induced in B-CLL cells. On light microscopy, cells exposed to medium, A23187, IL-2, BCDF, or T24 alone became only slightly enlarged but remained for the most part round. TPA induced the follow-

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**Table 2. Immunophenotypes of Patients Studied (Before T Cell Depletion)**

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*References for reagents: RF reagents, FMC7, Tac.*


§Polyclonal goat antihuman antisera; all others murine MoAbs.

#SmIg was expressed as undetectable (Und); monoclonality could be demonstrated by enzyme-linked immunosorbent assays (ELISA) of the treated cells by measuring the Ig produced (patient 4). IgM; patient 5, IgG; patient 6, IgM; patient 9, IgM; patient 12, IgG.
ing morphological alterations: increase in cell size; elongation of the clear and occasionally basophilic cytoplasm; and often bizarre, irregular cell forms. Cells treated with the double stimulus of TPA and A23187 increased in size but remained round or oval, the nuclei were eccentrically located, and the cytoplasm became deeply basophilic with a pronounced perinuclear clear zone. Combinations of TPA or TPA plus A23187 with other reagents did not result in different (or increased) morphological changes other than those described for these stimuli.

The vast majority of cells per well (70% to 90%) displayed the described morphological features after incubation with the respective agents. Less than 1% of the cells in any of the cultures treated with different inducers showed mitosis on cytopsin slide preparations. In addition, less than 1% were positive for the cell cycle marker Ki-67 in immunofluorescent assays.

Figure 1 depicts a comparison of cell sizes after treatment with different inducers in a representative case. Only small alterations in cell size were induced by A23187, BCDF, T24, or IL-2. Again, combinations of TPA and A23187 led to a substantial increase in cell size.

Analysis of Tac expression in cultured B-CLL cells. Most samples (except those from patients 11, 13, and 15) were negative or only marginally positive after staining with Tac MoAb. After culture for three days with the double stimulus of TPA plus A23187, 45% (±21%) of B cells from the eight CLL patients tested expressed IL-2 receptors as detected by flow cytometric analysis (Fig 2). Medium, TPA, A23187, IL-2, T24, and BCDF alone also induced IL-2 receptor, albeit at low levels (10% to 30%, which might be due to the culture conditions when using 10% FCS).

To our knowledge, the putative BCDF (IL-6) receptor has not yet been isolated. MoAbs detecting the BCDF receptor have therefore not been available for this study.

RNA synthesis caused by induction of B-CLL cells. In all cases, the RNA synthesis (measured by 3H-uridine incorporation) was significantly higher in TPA- or TPA plus A23187–exposed cultures than in wells incubated with medium, A23187, BCDF, T24, or IL-2 alone or with different combinations of these reagents (Fig 3). In all but one sample (patient 16), TPA and A23187 acted synergisti-
cally in inducing RNA synthesis. The further addition of other inducers (BCDF, T24, or IL-2) to TPA or TPA plus A23187 did not have significant effects on 3H-uridine uptake.

Ig production in B-CLL cells treated with inducing stimuli. Normal peripheral blood B lymphocytes from three normal donors responded strongly to BCDF, T24, and IL-2, whereas TPA and TPA plus A23187 were not effective (Fig 4). Coculture with irradiated T cells amplified these results by a factor of 2 to 3 (data not shown).

In parallel with the change in morphology and uptake of 3H-uridine, an increase in the amounts of IgM produced was found when using TPA or TPA plus A23187 in B-CLL cells (Fig 5). In contrast, no significant IgM production was seen in the control, A23187-, BCDF-, T24-, or IL-2-treated cultures during the same period. The two patients who produced IgG showed very similar results. Repeated or delayed addition of IL-2 or BCDF or different amounts of IL-2 (from 50 to 250 units, in most cases 250 units were applied) had no positive effects.

Although the response was variable, cells from all patients produced increased levels of Ig in the presence of TPA or TPA plus A23187. In all SmIgM-positive cases this was predominantly IgM, with little or no IgG production. For 11 patients the magnitude of Ig production in response to the doublestimulus of phorbol ester and calcium ionophore was significantly greater than that produced by the same leukemic B cells treated with TPA alone. It is not known why in the other cases the coculturing with TPA and A23187 was not effective in a synergistic fashion. Two representative examples for this phenomenon are shown in Fig 6. No obvious correlation with clinical, morphological, or immunologic features could be detected.

To examine whether the Ig was the product of leukemic (monoclonal) cells or derived from residual normal (polyclonal) B cells, cultures were also tested for monotypic light-chain expression. In each case only one light chain was detected and corresponded to the surface Ig heavy- and light-chain type (in those cases where it was detectable). In contrast, in the normal B cell cultures both IgM and IgG and both κ and λ light chains were produced.

The addition of purified autologous T cells to wells containing B-CLL cells did not result in the production of increased levels of IgM in any of the three patients tested (data not shown). When unfractionated leukemic cell populations (from three patients) were compared with the B-CLL populations from the same patient that were almost completely depleted of T cells (with less than 1% CD3+ cells), comparable amounts of Ig were produced.
As observed previously,36,37 leukemic B cells from some patients have the ability to produce large amounts of Ig spontaneously. In our study these patients (nos. 3 and 15) had B-PLL or B-CLL/PL. The morphological and functional properties of this type of leukemia have suggested that these cells represent a maturation arrest corresponding to that of physiologically activated B lymphocytes.22,38 When using the panel of inducing reagents these cells could be boosted to produce only slightly increased levels of Ig; here also, TPA and TPA plus A23187 were the most efficient stimuli, and IL-2 or BCDF had little effect.

Discussion

The maturation arrest seen in B-CLL and related leukemias reflects a defect in the ability to differentiate beyond a certain stage of maturation.38,39 In the last decade, there have been an increasing number of reports on differentiation induction experiments with B-CLL cells that can be induced to differentiate when provided with the appropriate signal.38,39

In a variety of cellular systems, the effector response to stimuli relies on signal transduction pathways using second messengers to activate effector kinases. We used the approach of induction of differentiation experiments to study the signal transduction mechanisms that appear to be impaired in B-CLL cells.

From data presented here and from previously reported experiments21 we made these main conclusions; (a) as described previously,1 CLL cells can be triggered by TPA to differentiate. (b) Although A23187 itself does not induce differentiation, it enhances synergistically the effect of TPA on the differentiation of CLL cells; the parameters used suggest maturation towards Ig-secreting cells. (c) Neither IL-2 nor BCDF (from peripheral blood B cells or from the T24 bladder carcinoma cell line) had any effect in the differentiation of CLL cells (singly or in combination with TPA and/or A23187) despite the expression of Tac antigen.

Individual variations in the response to TPA and/or TPA plus A23187 were seen in the series of samples tested. We could not establish which parameters influenced the extent of differentiation induced synergistically by phorbol ester and calcium ionophore and why in some cases the combination of these reagents was not more effective than TPA alone. Heterogeneity in the response of CLL cells to phorbol ester alone has already been documented previously.40 These authors suggested that differences in the response of B leukemic cells to TPA might reflect the underlying heterogeneity of the leukemic cells and might be correlated to slightly different stages at which the maturation arrest occurred.

Increased 3H-thymidine uptake and/or elevated Ig production has been observed in B-CLL cultures treated with IL-2 (IL-2 plus TPA, IL-2 plus anti-µ antibody, or IL-2 plus PHA), which has led a number of authors to suggest that B-CLL cells proliferate or differentiate in response to IL-2.41,45 However, it has not been shown unequivocally that the cells responding with differentiation belonged to the leukemic clone and not to residual normal B cells (for example by the demonstration of clonally restricted light-chain production).

The view that IL-2 can efficiently induce proliferation and/or differentiation in B-CLL cells has recently been challenged. Several groups reported that IL-2 is not able to promote the proliferation or differentiation of leukemic B cells.34,46-50 although IL-2 can induce significant proliferation and differentiation of activated normal B cells.51 Furthermore, the "H-thymidine uptake of B-CLL cells cannot necessarily be equated with "proliferation" because no increase in cell number or mitotic index has been reported.

Similarly, different B cell differentiation factors (including T24-BCDF) and other lymphokines (for example, interferons) were described to cause differentiation of B-CLL cells.34,44,52-54 Again, production of monoclonal light chains was not documented in any of the reports. Benjamin et al56 could clearly show that T and B cell-derived lymphokines influence the normal B cell response but not that of leukemic B cells. It is therefore difficult to assess whether groups observing differentiation in the presence of IL-2 or BCDF (IL-6) were in fact measuring responses in normal cells.

It is worth noting here that residual normal B cells or peripheral blood B cells from normal volunteers do not represent the normal counterparts to B-CLL cells (for the purpose of a "normal control") because these cells are polyclonal in surface immunoglobulin light- and heavy-chain expression and do not carry the CD5 antigen, which (although being a T cell marker) is a hallmark of B-CLL cells. At the level of visual immunofluorescence there is only a small minority of CD5-positive B cells in the normal peripheral blood.55

B-CLL populations produced quantitatively similar levels of IgM after stimulation with TPA or TPA plus A23187 whether T cells were present or had been removed. In contrast, induction of Ig production from normal B cells by these same agents was strongly T cell dependent. These data suggest that stimulation of differentiation in B-CLL cells by direct activation of PKC is T independent, whereas in normal B cells, differentiation may be stimulated directly but remains largely dependent on helper effects (soluble or cell mediated) that are provided by T cells.

Elements of the signal transduction mechanism downstream of and including PKC seem to be intact in B-CLL because the cells from all patients responded appropriately to direct stimulation by phorbol ester. The rise in intracellular Ca++ levels increased the potency of phorbol ester for inducing B-CLL cells to differentiate in 65% to 100% of the samples (depending on the parameters tested), thus indicating that that part of the protein phosphotransferase system that involves the second-messenger processing functions regularly in a coordinated process.

A recent publication57 suggested that PKC is also involved in IL-2-mediated signal transduction. By comparing the effects induced by agents that are known to directly activate PKC with the response seen after "physiological signal transduction" (upstream of PKC) with IL-2 and BCDF (IL-6) stimulation, we documented that the physiological ligands were unable to trigger differentiation in B-CLL cells, even though the signal transduction process evoked by extra-
cellular stimulants appears to be coordinated and effective in normal peripheral blood B cells.

Only Tac-positive normal B cells and not Tac-negative cells respond to IL-2 by differentiating into Ig-producing cells. B-CLL cells meet this precondition of expressing the IL-2 receptor. Thus, malignant B cell populations may spontaneously or after incubation with stimulants express the Tac antigen in the absence of a functional response to IL-2. This malfunction could be the result of an abnormality (chemical alteration?) of the IL-2 receptor or of a blockade of the signal between the IL-2 receptor and PKC.

Perri suggested that the defective expression of cell surface receptors for another B cell–directed lymphokine, B cell growth factor (BCGF), leads to an impaired responsiveness to BCGF by B-CLL cells. A similar defect might occur for the BCDF (IL-6) receptor. Alternatively, as proposed for IL-2, the processing of the initial signal given by BCDF–BCDF receptor interaction might be disturbed.

In the present study, we did not analyze the effects of anti-Ig antibodies of the B-CLL cells with regard to induced proliferation or differentiation. If RNA and DNA synthesis could be triggered by anti-Ig antibodies, then this might indicate that the signaling pathway of Slg receptors on B-CLL cells is in fact intact. Furthermore, because the phospholipid pathway is clearly not the only system used by lymphocyte receptors, this would suggest that different growth and differentiation factors use different messenger systems.

According to current dogma, members of a family of closely related guanosine triphosphate (GTP)-binding proteins (G proteins) are responsible for receptor-effector coupling in the transmembrane signaling. The activation of phospholipase C is G protein dependent. It has been shown that a variety of cell surface receptors that mediate growth, differentiation, secretion, etc., and that transmit their message by either the phosphatidylinositol-PKC system or through a second protein phosphotransferase system associated with the cAMP-dependent protein kinases, do so through a complex mechanism involving stimulatory (G) and inhibitory (Gi) activities of two different G proteins.

Recent data suggest that the IL-2 receptor mediates signal transduction via a GTP-binding protein. This family of regulatory proteins that appear to represent crucial initial events for a variety of signal transduction reactions attracted much interest when it was described that GTP-binding proteins might be encoded by ras oncogenes. Further information on the G protein control of the transmembrane signaling linked to PKC activation will allow a more thorough evaluation of possible defects in the signal transduction that are possibly located in this part of the pathway.

In conclusion, the data demonstrate that commitment to the differentiation program can be initiated efficiently in B-CLL cells by phorbol ester and calcium ionophore acting together. The findings suggest that PKC activation by a direct signal delivered by TPA and A23187 leads to distinct responses in B-CLL cells that are characteristic of maturation towards plasma cells. The results indicate that the second-messenger system is not involved in any malfunction of the signal transduction system. On the other hand, a physiological response to the extracellular stimulants IL-2 and BCDF (IL-6) was not observed, which suggests that the molecular control of differentiation that affects the signal transduction at or immediately after ligand-receptor interaction might be impaired. It therefore appears that early signal transduction events (upstream of the activation of the key enzyme PKC in the cascade of signal transmission) might function incorrectly or not at all. The nature of this defect remains to be elucidated. Our data do not exclude that other growth or differentiation factors might use different messenger systems that might be intact in B-CLL cells.

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