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SYNERGISTIC ACTION OF A23187 AND PHORBOL ESTER ON ACTIVATION OF B CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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INTRODUCTION

Monoclonal malignancies of lymphocytic origin provide an excellent model for the investigation of regulatory aspects of cellular growth and differentiation. In chronic lymphocytic leukemia (CLL) monoclonal peripheral B cells are restricted within an early maturational stage. CLL-B cells can be stimulated to proliferate and to differentiate into Ig-producing cells by various mitogens and/or T cell derived factors (1-5). One of the most potent stimulators of T and B cell proliferation, the tumor promoting phorbol ester phorbol-12-myristate-13-acetate (6,7), has been reported to stimulate terminal differentiation of CLL-B cells (8).

It has become evident that calcium ionophores synergize with PMA with respect to various cellular functions such as the release of bioactive factors by platelets, mast cells and neutrophils (9), and the proliferation of murine thymocytes and human T cells (10,11). We recently described the existence of a similar synergism in human B cells with respect to proliferation, to the expression of several activation markers and to responsiveness to B Cell Differentiation Factor (BCDF) (12). These observations led us to investigate the effects of the calcium ionophore A23187 and PMA on several aspects of activation of CLL-B cells.

MATERIALS AND METHODS

Preparation of cells. Mononuclear cells (MNC) were obtained from six B-CLL patients. T cells were removed by sheep erythrocyte rosetting. Preparations then contained less than 0.25% T cells as judged by OKT11 immunofluorescence. The cells were cultured in 96-well flat-bottom plates at $2x10^5$ per well. ^{3}H-thymidine incorporation was determined on day 4 of culture. Fluorescence assays for the detection of the B cell activation markers Tac, Transferrin receptor and HLA-DR were performed on day 3 according to standard procedures. Cells samples were analyzed on an Ortho-Cytofluorograph. A spot-ELISA was used to enumerate Ig secreting B cells (13). Briefly, stimulated CLL-B cells were incubated in 96-well plates coated with anti human IgM. After removal of the cells, individual wells were incubated with alkaline phosphatase-conjugated

anti IgM. The substrate 5-bromo 4-chloro 3-indolyl phosphate was added in agarose. Individual antibody secreting cells were represented as blue spots.

Anti Tac ascites was kindly provided by Dr. T. Waldmann. The human T cell hybrid Ko 2.9 was used as a source of BCDF (T. Logtenberg, manuscript in preparation).

RESULTS AND DISCUSSION

PMA at 10 ng/ml induced proliferation of CLL-B cells, whereas the ionophore A23187 was not mitogenic for these cells. However, combination of the ionophore with non-mitogenic concentrations of PMA (1-3 ng/ml) resulted in vigorous proliferation (Fig. 1), extending our results with normal resting B cells (12). In one case the cells failed to respond to any of the combinations used.

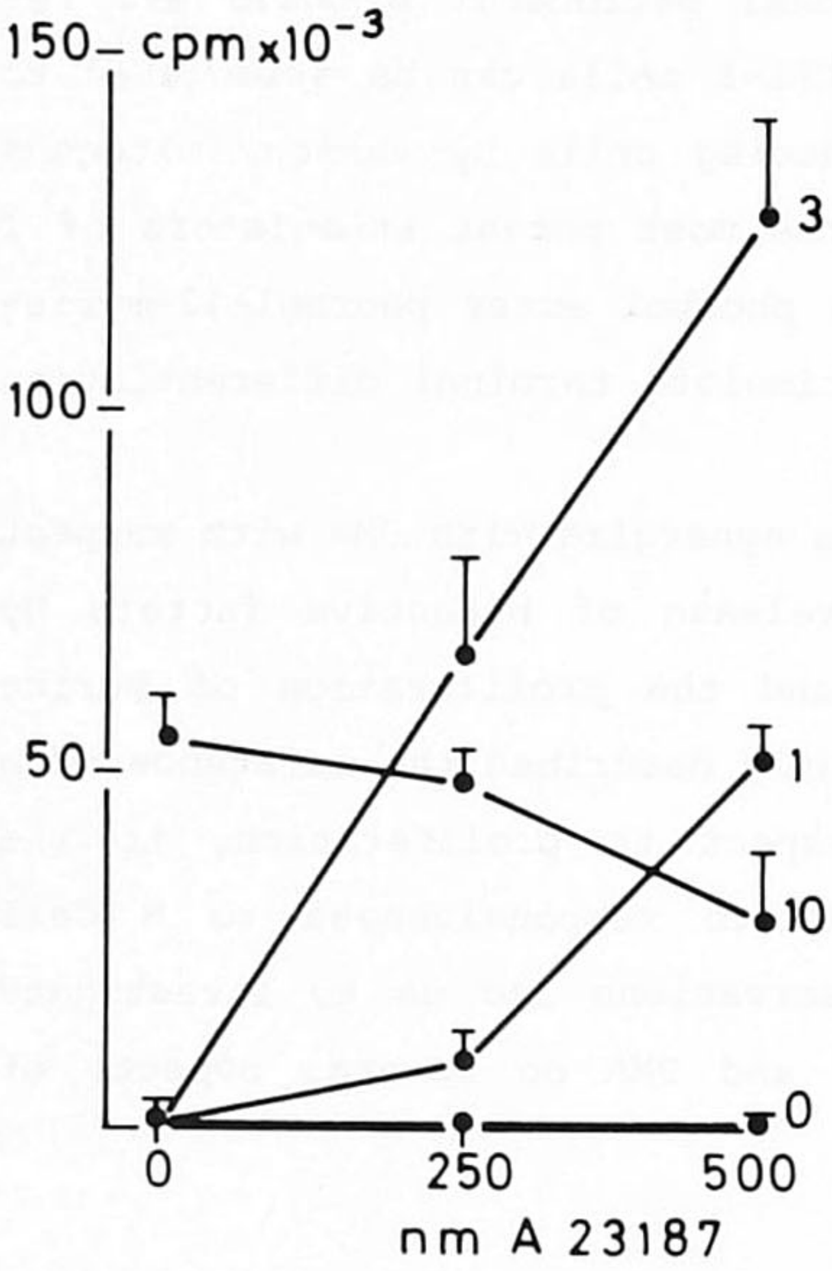

Fig. 1 - Proliferation of CLL-B cells (patient Kn) in the presence of A23187 (0, 250, 500 nM) and/or PMA (0, 1, 3, 10 ng/ml)

The expression of three surface activation markers was determined after a three day stimulation with PMA and/or A23187 at concentrations that, in combination, induced optimal proliferation. Fig. 2 depicts the results of FACS analysis in a representative case. In all patients ionophore A23187 did not modify the expression of the markers as compared to the unstimulated control cultures. PMA induced a slight increase only in HLA-DR expression. Combination of the two agents strongly enhanced the effect of PMA on this marker and induced the appearance of Tac antigen and the Transferrin receptor. Fig. 3 depicts axial light loss in a representative case. PMA and A23187 at the indicated concentrations only induced small alterations in cell size. Again, combination of the agents led to a strong increase in this parameter.

Since PMA at high concentrations (> 100 ng/ml) has been reported to induce differentiation of CLL-B cells into plasma cells (8), we also studied Ig production and the responsiveness to BCDF. To that end cells of each of the six patients were cultured for 5 days in the presence of different combinations of A23187 and PMA. Alternatively, cells were stimulated with PMA and A23187 for 1 day, followed by a 4 day incubation in the presence of supernatant from the BCDF-producing human T-hybridoma Ko 2.9. A spot-ELISA specific for IgM was used

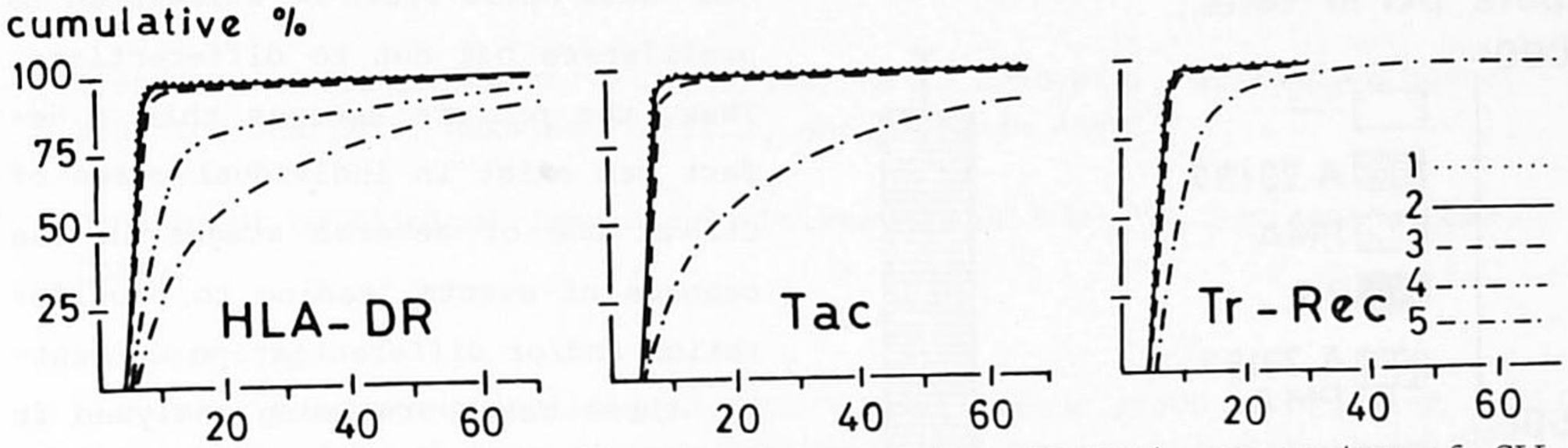

Fig. 2 - HLA-DR, Tac and Transferrin receptor (Tr-Rec) expression of CLL-B cells (patient Kn) after a 3 day culture period. 1 = unstained control; 2= unstimulated; 3= 500 nM A23187; 4= 3 ng/ml PMA; 5 = 3+4. Results are presented as the cumulative % of relative fluorescence on a linear scale.

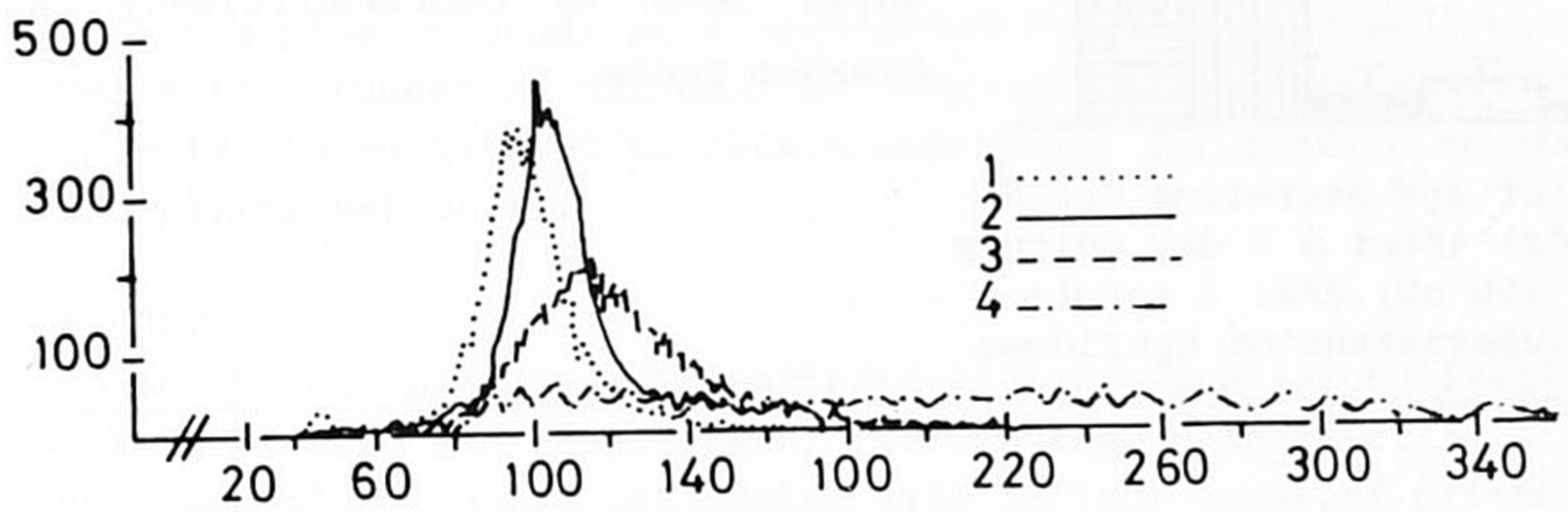

Fig. 3 - Axial light loss (correlating with cell size) of CLL-B cells (patient Kn) on day 3 of culture. 1 = unstimulated; 2 = 500 nM A23187; 3 = 3 ng/ml PMA; 4 = 2+3.

as a read-out system. Fig. 4 depicts the results obtained in a representative case. The combination of PMA and A23187 resulted in Ig production in a substantial number of cells in 4 of 6 cases. The addition of BCDF further increased numbers of Ig secreting cells, confirming that CLL-B cells respond to T cell derived regulatory signals.

An important early event in the activation of many cell types is the hydrolysis of phosphorylated derivatives of PI, resulting in the formation of inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 is the hypothetical mediator of the rise in free cytosolic Ca^{++}. DAG has been shown to activate the calcium- and phospholipid-dependent enzyme protein kinase C by increasing its affinity for Ca^{++}. This enzyme is also activated by PMA (9). Calcium ionophores, such as A23187, and PMA allow for the separate investigation of each of the two branches of this bifurcating mechanism. Recent reports indicate that this messenger system is linked to membrane Ig in B lymphocytes (14). Here we demonstrate that both pathways are operative in proliferation and in differentiation of CLL-B cells. Different responses, however, can occur: in one case no change in any of the parameters studied could be induced. Furthermore, in a sec-

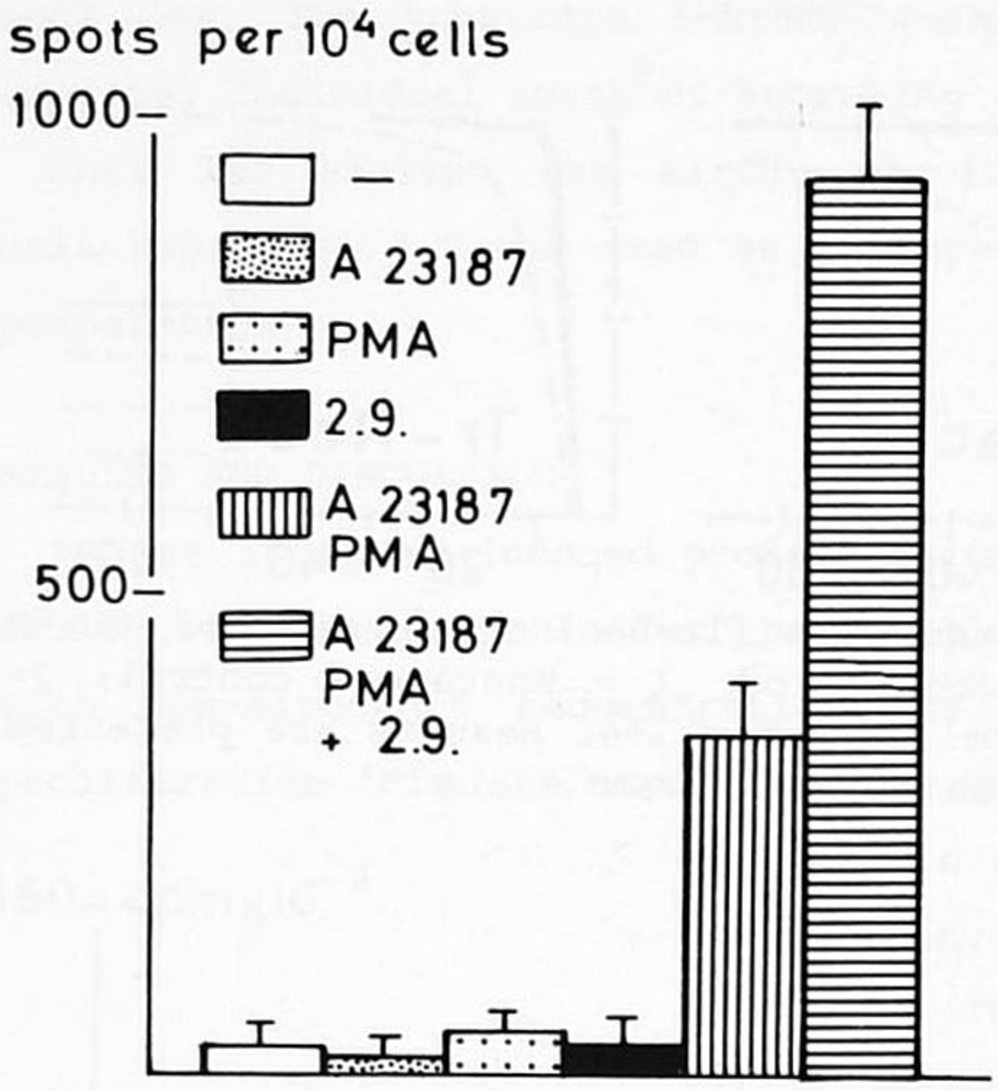

Fig. 4 - Number of IgM secreting CLL-B cells (patient Cr) after a 5 day culture period. A23187: 250 nM; PMA: 1 ng/ml; 2.9: 10% (v/v) supernatant of hybridoma Ko 2.9.

ond case cells could be stimulated to proliferate but not to differentiate. Thus, the picture emerges that a defect can exist in individual cases of CLL at one of several stages in the cascade of events leading to proliferation and/or differentiation.Currently, these cases are being analysed in order to "map" these defects in the activation cascade. We feel that this strategy will lead to a better understanding of the diverse mechanisms which lead to immunodeficiency in a broader sense.

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