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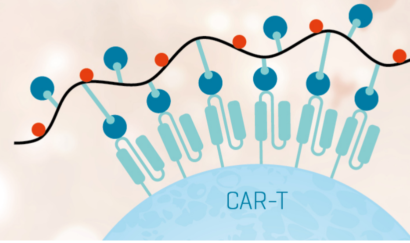

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INDUCTION OF DNA FRAGMENTATION IN CHRONIC B-LYMPHOCYTIC LEUKEMIA CELLS[1]

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Chronic lymphocytic leukemia of B cell type (B-CLL) is a neoplastic disorder characterized by the accumulation of small resting lymphocytes in the periphery. The phenotype of these cells suggests that they are "frozen" at an early stage of maturation. Glucocorticoid hormones are commonly used to treat patients with B-CLL, resulting in a reduction in the peripheral lymphocyte count by an undefined mechanism. Here we report that glucocorticoids stimulate DNA fragmentation characteristic of a suicide process known as apoptosis or programmed cell death (PCD) in suspensions of cells from patients with B-CLL. The effects can be mimicked by Ca^{2+} ionophore and involve a sustained increase in the cytosolic Ca^{2+} concentration. Specific antibodies binding to membrane-associated IgM on the leukemic cells can also induce PCD by a similar mechanism. Phorbol esters block DNA fragmentation and cell killing in response to all of the agents, suggesting that activation of protein kinase C desensitizes the cells to PCD. Targeting the B-CLL cells with antibodies that induce an unbalanced, sustained Ca^{2+} increase may therefore represent a rational strategy for the destruction of leukemic cells.

Apoptosis, or PCD,[3] is a process of cell deletion that occurs in response to a number of physiologically relevant stimuli. The process is characterized by several early morphologic alterations, including plasma and nuclear membrane blebbing, organelle relocalization, and chromatin condensation (1). Endogenous endonuclease activation, resulting in the cleavage of host chromatin into oligonucleosome-length DNA fragments (2), is the most characteristic biochemical marker for PCD and may directly precipitate cell death (3).

Most of our knowledge about the mechanisms underlying apoptosis comes from studies with immature thymocytes, which readily undergo PCD in response to glucocorticoid hormones (2, 4) or Ca^{2+} ionophores (3, 5, 6). Our recent work has shown that thymocyte apoptosis is dependent on an early, sustained increase in the cytosolic Ca^{2+} concentration, which directly mediates endonuclease activation and cell death (3, 7, 8). Antibodies to the CD3/T cell Ag receptor complex also induce PCD in thymocytes (6, 9, 10) by an analogous Ca^{2+}-dependent mechanism (9). A possibly related phenomenon known as "activation-induced cell death" has been identified in T cell (11, 12) and B cell (13, 14) lymphomas. It has been proposed that PCD in response to Ag receptor signaling may be involved in the induction of T cell tolerance to self-Ag in the thymus.

A recent study has linked DNA fragmentation to the effects of glucocorticoids in patients with acute lymphoblastic leukemia and chronic myeloid leukemia in vivo (15). We therefore asked whether induction of apoptosis could be involved in the therapeutic effects of glucocorticoid hormones in patients with B-CLL. Here we report that glucocorticoid hormones, Ca^{2+} ionophore, and anti-IgM antibodies can induce PCD in B-CLL cells and discuss how this property might be exploited in therapy.

MATERIALS AND METHODS

Cell isolation and incubation. PBL were isolated from healthy individuals or patients diagnosed for B-CLL before they had undergone treatment. B-CLL cells and normal B cells from tonsil tissue were purified by negative selection by using two cycles of T cell rosetting as described previously (16). Thymus glands isolated from patients under 2 yr old undergoing corrective surgery were used to prepare thymocyte suspensions, as described previously (9). Cells were incubated at a concentration of $10 \times 10^6 \times ml^{-1}$ in RPMI 1640 medium supplemented with 1% (w/v) BSA in a humidified incubator (37°C) under an atmosphere of 5% CO_2 in air. Viability was determined by trypan blue exclusion and was routinely 90 to 100% after the isolation procedures. Normal B cells were activated by preincubation with heat-inactivated streptococcal cells (Pansorbin) for 24 h.

Agarose gel electrophoresis of DNA fragments. Thymocytes and B-CLL cells were incubated in the RPMI medium for 18 h with the agents indicated. Thereafter, cells (50×10^6) were harvested by centrifugation for 30 s at $1500 \times g$ in a microcentrifuge, resuspended in 0.5 ml PBS and lysed by addition of 2 volumes of a lysis buffer containing 10 mM Tris, 20 mM EDTA, and 0.5% Triton X-100, pH 8.0. Lysed samples were centrifuged for 20 min at $13,000 \times g$ to separate intact chromatin (pellet) from DNA fragments (supernatant) (4, 9). Supernatant fractions were extracted with equal volumes of phenol, phenol/chloroform 1/1, and chloroform, precipitated with 2 volumes absolute ethanol containing 0.15 M NaCl, and resuspended in 20 μl of a buffer containing 20 mM Tris, 1 mM EDTA, and 0.5% SDS, pH 8.0. Samples were electrophoresed for 2 h at 50 V in 1.8% agarose gels preimpregnated with 0.5 mg $\times$ ml^{-1} ethidium bromide, and DNA was visualized by UV light.

Quantitation of DNA fragmentation. Cells ($10 \times 10^6 \times ml^{-1}$) or nuclei were incubated with the agents indicated. Aliquots of 1 ml were lysed with 0.5 ml of the lysis buffer described above and centrifuged for 20 min at $13,000 \times g$ to separate intact chromatin (pellet) from DNA fragments (supernatant). DNA content in pellet and supernatant fractions was determined by using the diphenyl-

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[3] Abbreviations used in this paper: PCD, programmed cell death; B-CLL, chronic B-lymphocytic leukemia; fura-2 AM, fura-2 tetraacetoxymethyl ester; PKC, protein kinase C.

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amine reagent (17). Results are expressed as the percentage of total DNA in each sample that resisted sedimentation at 13,000 × *g*.

Calcium-dependent DNA fragmentation in isolated nuclei. Nuclei from B-CLL lymphocytes, thymocytes, and PBL were isolated as described previously (5) and resuspended by homogenization in a "TKM" buffer containing 10 mM Tris, 150 mM KCl, 10 mM $MgCl_2$, 1 mM NAD^+, and 1 mM ATP, pH 7.2. After incubation, 1-ml aliquots were lysed, DNA fragments were separated from intact chromatin by centrifugation, and DNA content was determined by using the diphenylamine reagent. Results are expressed as the percentage of total DNA in each sample that resisted sedimentation at 13,000 × *g*.

Measurement of cytosolic Ca^{2+} concentration. Cells were loaded with 0.5 μM fura-2 tetra-acetoxymethyl ester for 45 min at 25°C (anti-IgM experiments) or 37°C (methylprednisolone experiments). One-milliliter aliquots were washed by centrifugation for 30 s at 1,500 × *g* in a microcentrifuge, resuspended in 2 ml of the (37°C) RPMI medium, and cytosolic Ca^{2+} concentrations were determined as described previously (9, 18). Addition of 20 mM EGTA to the washed cells resulted in no change in fluorescence intensity, confirming that washing removed all extracellular dye. Methylprednisolone did not detectably alter cell autofluorescence in unloaded cells. Incubation of untreated cells did not alter the fluorescence in fura-2-loaded cells.

Materials. PMA, fura-2 AM, ethidium bromide, methylprednisolone, and the Ca^{2+} ionophore A23187 were purchased from Sigma Chemical Co. (St. Louis, MO). RPMI 1640 medium was from GIBCO (Paisley, Scotland). mAb (0.1 × ml^{-1} stocks) were obtained from Dakopatts a/s (Copenhagen, Denmark), and heat-inactivated streptococcal cells (Pansorbin) from Calbiochem (San Diego, CA).

RESULTS

DNA fragmentation in B-CLL cells. Glucocorticoid hormones and calcium ionophores are known to induce PCD in immature thymocytes (3–7, 9). Both induced extensive chromatin cleavage in several B-CLL cell populations, the frequency of responding cell samples being higher for methylprednisolone than for the calcium ionophore, A23187 (Table I). Normal isolated tonsillar B cells also exhibited sensitivity to both agents (Table II). In cases 6 and 7, activating the normal B cells made them resistant to glucocorticoid, whereas ionophore sensitivity was unchanged. Daudi B cell clones were resistant to the lytic effects of both agents (not shown). In the responsive samples agarose gel electrophoresis revealed a DNA degradation pattern similar to that observed in steroid-treated thymocytes (Fig. 1), and the dose dependencies for glucocorticoid or ionophore were also quite similar (Fig. 2).

Because anti-CD3 mAb stimulate PCD in isolated thymocytes (9), thymic lobes (6), and in vivo (10), the observation that glucocorticoid or ionophore induced DNA degradation in thymocytes and some B-CLL populations suggested that analogous activating mAb might also stimulate PCD in the latter. Phenotypic analysis of the B-CLL cells revealed high IgM expression, consistent with previous observations (19). Anti-IgM mAb induced both DNA fragmentation (Table I) and cell death (Tables III and IV) in B-CLL cells. Other mAb were not effective in the absence of cross-linking (Table III). Agarose gel electrophoresis revealed a clear "ladder" DNA degradation pattern characteristic of PCD (Fig. 1).

TABLE I

Effects of calcium ionophore, methylprednisolone and anti-IgM antibodies on DNA fragmentation in B-CLL cells[a]

Case	Effect on % DNA Fragmentation			
	Control	Ionophore	Methylprednisolone	Anti-IgM
1	0	14	45	47
2	9	3	10	9
3	10	38	46	42
4	9	9	21	13
5	10	11	34	46
6	14	20	44	20
7	8	34	38	50
Sensitive[b]		3/7	6/7	4/7

[a] B-Cell cells were treated with 1 μM of calcium ionophore A23187 or methylprednisolone and with 8 μg/ml of monoclonal anti-IgM for 24 h, and DNA fragmentation was measured as described in *Materials and Methods*.

[b] More than 50% above control.

TABLE II

Effect of calcium ionophore, methylprednisolone, and anti-IgM antibodies on DNA fragmentation in normal B cells[a]

Case	Activation[b]	Effect on % DNA Fragmentation			
		Control	Ionophore	Methylprednisolone	Anti-IgM
1	−	18	15	21	13
1	+	16	ND	ND	15
2	−	8	7	10	10
3	−	9	40	30	18
4	−	17	31	25	15
5	−	12	29	ND	14
6	−	16	34	26	13
6	+	16	29	17	13
7	−	7	23	24	7
7	+	13	27	11	16
Sensitive[c]			5/9	2/8	0/10

[a] Tonsillar B cells were isolated and incubated with the agents indicated for 24 h before determination of DNA fragmentation, as described in *Materials and Methods*.

[b] Activated by heat-inactivated streptococcal cells (Pansorbin) for 24 h before testing.

[c] More than 50% above control.

Evidence for the involvement of calcium. Our previous work has shown that thymocyte apoptosis is often dependent on a sustained cytosolic calcium increase; we therefore investigated whether calcium was also involved in DNA fragmentation in the B-CLL cells. Methylprednisolone induced a sustained calcium increase in the B-CLL cells loaded with the dye fura-2 AM (Fig. 3). The steroid antagonist RU-486 (7) blocked the increase, suggesting that the mechanism involved steroid receptor binding. Glucocorticoid-induced thymocyte apoptosis is dependent upon macromolecular synthesis (4, 5), which may be linked to the production of a cytosolic factor that facilitates calcium influx (7). Cycloheximide, a potent inhibitor of protein synthesis, blocked the glucocorticoid-stimulated calcium increase in B-CLL cells (Fig. 3), indicating that a similar mechanism may have been involved.

The soluble anti-IgM mAb also induced an increase in the cytosolic calcium level in the B-CLL cells (Fig. 4*A*) that was sustained for at least 2 h (250 plus or minus 15 nM in the anti-IgM-treated cells vs 90 plus or minus 5 nM in controls; mean plus or minus SD, $n = 3$). Stimulation of cells in a calcium-depleted medium resulted in a transient calcium increase (Fig. 4*B*), indicating that a portion of the initial increase was caused by release from intracellular stores and that the sustained component was dependent on calcium influx. This pattern of calcium mobilization is analogous to that observed in response to Ag receptor stimulation of thymocytes (9).

The chromatin cleavage observed in apoptotic thymocytes has been linked to the activation of an endogenous, nuclear endonuclease. This activity can be readily detected in nuclei isolated from untreated thymocytes (4, 5) or rat liver (20) reconstituted with calcium and magnesium, but not in nuclei from some other cellular sources (4). Nuclei isolated from the B-CLL cells, but not PBL or Daudi cells (not shown), also exhibited substantial calcium-dependent endogenous endonuclease activity

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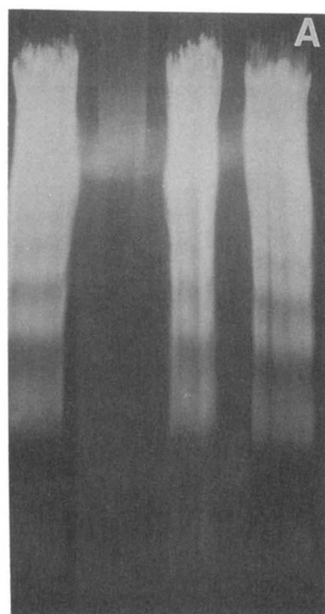

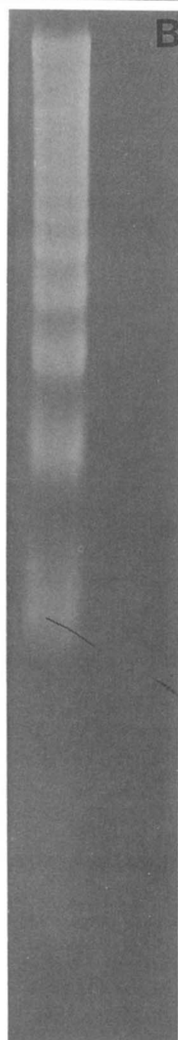

Figure 1. DNA fragmentation in B-CLL lymphocytes exposed to glucocorticoid or Ca^{2+} ionophore. *A*, B-CLL cells or thymocytes were incubated with or without 1 μM methylprednisolone or 400 nM Ca^{2+} ionophore A23187 for 18 h at 37°C. DNA fragments were isolated and resolved by agarose gel electrophoresis, as described in *Materials and Methods*. *Lane 1*, thymocytes plus methylprednisolone; *lane 2*, untreated B-CLL lymphocytes; *lane 3*, B-CLL lymphocytes plus methylprednisolone; *lane 4*, B-CLL lymphocytes plus calcium ionophore A23187. *B*, B-CLL cells were incubated for 6 h with a 1/200 dilution of anti-IgM mAb before isolation of DNA fragments and electrophoresis. *Lane 1*, B-CLL cells plus anti-IgM mAb.

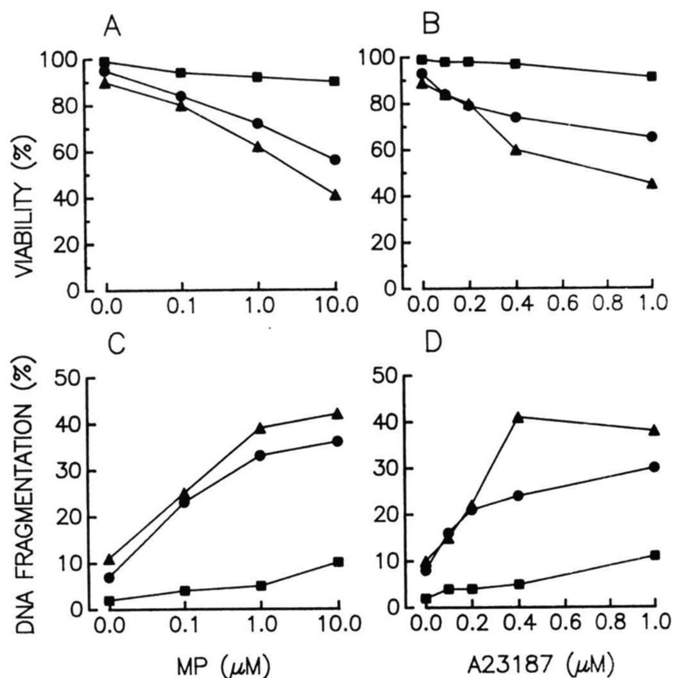

Figure 2. Glucocorticoid- and Ca^{2+} ionophore-induced DNA fragmentation and cell killing in thymocytes, B-CLL cells, and normal lymphocytes. Cells were incubated with the indicated concentrations of methylprednisolone (*MP*) or the Ca^{2+} ionophore A23187. Viability was determined by trypan blue exclusion. Results are from one experiment characteristic of four replicates. ●, B-CLL lymphocytes; ▲, thymocytes; ■, normal lymphocytes.

TABLE III

Antibody-induced DNA fragmentation is selective for anti-IgM[a]

Antibody	Cytosolic Ca^{2+} (nM)	DNA Fragmentation (%)	Viability (%)
IgM	690	42	60
CD5	90	14	83
CD19	85	16	80

[a] Isolated B-CLL cells were incubated with a 1/100 dilution of each mAb. Greater than 80% of the cells bound the mAb. The maximum increase in cytosolic Ca^{2+} level and DNA fragmentation and cell viability after 18-h incubation was determined as described in *Materials and Methods*. Average results from two separate experiments.

TABLE IV

Inhibition of cell killing in response to methylprednisolone, Ca^{2+} ionophore, and anti-IgM antibody by PMA[a]

Treatment	Viability (%)	
	Without PMA	With 10 nM PMA
Control	92	81
MP, 1 μM	42	82
Ionophore A23187, 400 nM	47	95
Anti-IgM, 1/200	61	96

[a] Isolated B-CLL cells were incubated with methylprednisolone, Ca^{2+} ionophore A23187, or anti-IgM mAb and cell viability was determined by trypan blue exclusion after 18 h. Data represent viable cells. One representative experiment.

(Table V).

Phorbol ester blocks PCD. Phorbol esters (9, 21) and IL-1 (22) inhibit PCD in thymocytes by mechanisms that appear to involve activation of PKC. Phorbol esters also blocked both DNA fragmentation and cell death in human synovial cells exposed to cold shock (23). The phorbol ester PMA inhibited both DNA fragmentation (Fig. 5) and cell death (Table V) in response to glucocorticoid, iono-

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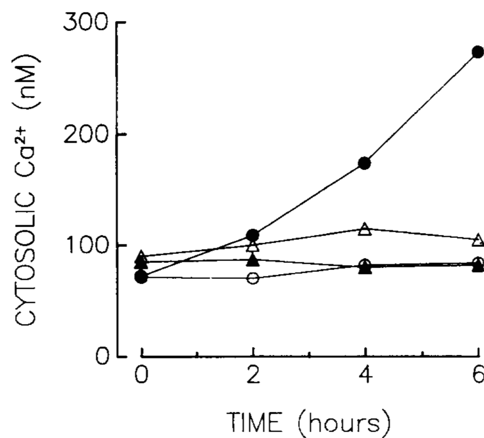

Figure 3. Glucocorticoid increases the cytosolic Ca^{2+} concentration in B-CLL lymphocytes. Results of one experiment characteristic of three replicates. ○, Control; ●, 1 μM methylprednisolone; ▲, methylprednisolone plus 10 μM RU-486; △, methylprednisolone plus 10 μM cycloheximide.

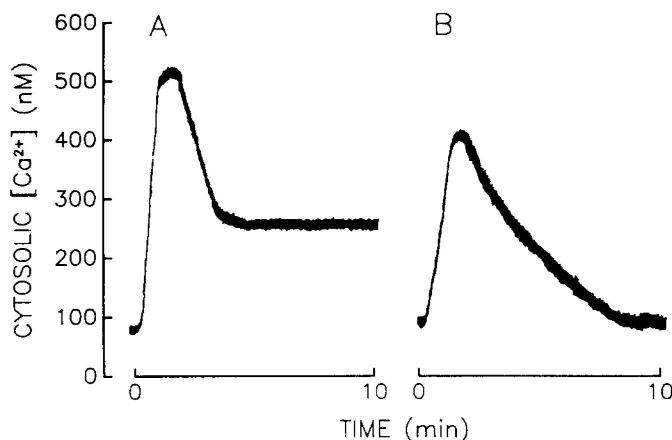

Figure 4. Anti-IgM mAb stimulates an increase in the cytosolic Ca^{2+} concentration in B-CLL cells. *A*, Fura-2-loaded cells (10×10^6) in RPMI 1640 medium without BSA were stimulated with a 1/200 dilution of the antibody in a water-jacketed (37°C) cuvette. *B*, Fura-2-loaded cells (10×10^6) stimulated with anti-IgM mAb in RPMI 1640 medium without BSA containing 10 mM EGTA. Cytosolic Ca^{2+} concentration was determined as described previously (16). Results are direct reproductions of fluorescence traces. Data from one experiment that was typical of six replicates.

TABLE V
Calcium-dependent DNA fragmentation in isolated B-CLL cell nuclei[a]

Cell Source	% DNA Fragmentation	
	5 mM EGTA	5 mM $CaCl_2$
B-CLL	6 ± 2	42 ± 5
Thymocytes	5 ± 1	38 ± 4
PBL	2 ± 0.5	5 ± 1

[a] Isolated nuclei were incubated in the TKM buffer (which contains 10 mM Tris, 150 mM KCl, 10 mM $MgCl_2$, 1 mM NAD^+, and 1 mM ATP, pH 7.2) with 5 mM EGTA or 5 mM $CaCl_2$ for 60 min at 37°C before determination of DNA fragmentation, as described in *Materials and Methods*. Mean ± SD, $n = 3$.

phore, or anti-IgM mAb. Thus, PKC activation may also block PCD in B-CLL lymphocytes.

DISCUSSION

The results of several recent studies have generated interest in the potential for exploiting the sensitivity of certain neoplastic cells to apoptosis in cancer therapy. Chromatin cleavage occurs spontaneously in cells from children with acute T-lymphoblastic leukemia in vivo (24) and is induced by glucocorticoids in several types of leukemic cells (15). Chromatin condensation and cleavage occur spontaneously in B-CLL lymphocytes cultured for extended periods in vitro (25). Anti-IgM mAb induce activation-induced cell death, a particular PCD response, in B cell lymphomas (13, 14). More recently, Trauth and co-workers have shown that a particular anti-idiotype mAb (APO-1) can induce tumor regression by induction of apoptosis in leukemic cells in nude mice (26), which may be related to the results of earlier work demonstrating anti-tumor effects induced by other anti-idiotype antibodies (27, 28). The present findings support and extend these observations by showing that the mechanism for the DNA fragmentation observed appears to involve a sustained calcium increase.

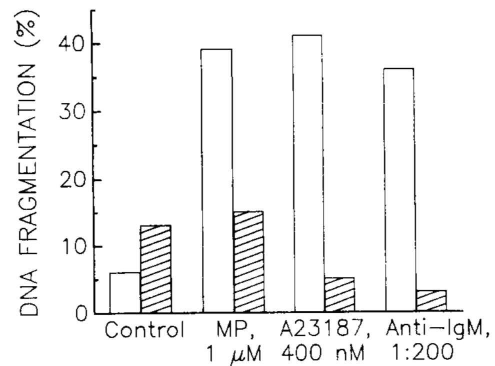

Figure 5. Phorbol ester inhibits DNA fragmentation in B-CLL cells treated with glucocorticoid, Ca^{2+} ionophore, or anti-IgM mAb. Cells were incubated 18 h with the indicated concentrations of methylprednisolone (*MP*), the Ca^{2+} ionophore A23187, or anti-IgM antibody in the absence (*open bars*) or presence (*hatched bars*) of 10 nM PMA, and DNA fragmentation was quantitated as described in *Materials and Methods*. Results are averages from four experiments; SD did not exceed 10% of mean values.

Normal tonsillar B cells also showed some sensitivity to glucocortocoid- or ionophore-induced PCD. We speculate that this is caused by the presence of apoptosis-sensitive immature B cells (centrocytes) within the population (29); however, neither cells from a model B cell line nor PBL were responsive. The basis for the observation that tonsillar B cells are somewhat sensitive to PCD whereas peripheral B cells are not is at present unknown. Possibly relevant is the observation that compartmentalized immature (thymocytes) or mature (splenic) T cells contain endogenous nuclear endonuclease activity constitutively (4), whereas peripheral T cells do not (4) (present study). Importantly, we also detected a calcium-dependent endonuclease activity in isolated nuclei from B-CLL cells. The presence or absence of this constitutive activity may account for the effects observed in intact cells.

Apoptosis is an extremely selective type of cell deletion; apoptotic cells often exist directly adjacent to unaffected neighboring cells. This selectivity is one reason for the current interest in PCD as a potential mechanism for tumor regression. The observation that glucocorticoids induce DNA fragmentation in leukemic cells (15) (present study) may be relevant to their effectiveness as antineoplastic agents. However, glucocorticoids also induce PCD in immature T and B cells, and their specificity is therefore not ideal. In contrast, anti-IgM mAb stimulated DNA fragmentation only in B-CLL cells, with no detectable effects on tonsillar B cells, PBL, or Daudi B cell clones. Therefore, treatment with more specific anti-tumor mAb may better exploit the target specificity of PCD, although

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the clinical efficacy of this approach remains to be established.

Our observation that anti-IgM mAb induce apoptosis in B-CLL cells stands in apparent contrast to the previous finding that anti-Ig can actually rescue a B cell subset from undergoing apoptosis (29). We cannot explain this discrepancy at present, although it is likely to result from the fact that normal and neoplastic cells are inherently different. However, the general conclusion that agents that can activate a particular cell type can also block apoptosis is completely consistent with the findings of the present study; phorbol ester blocked PCD in the B-CLL cells, and activation of tonsillar B cells inhibited methylprednisolone-induced DNA fragmentation. Just as anti-CD3 mAb can induce proliferation or death, depending on the T cell type, it is possible that anti-Ig can induce qualitatively different signaling in normal B cells with respect to the neoplastic B-CLL cells, and this difference may underlie the discrepancy.

Not all populations of B-CLL lymphocytes were found to be equally sensitive to the effects of glucocorticoid, ionophore, or mAb treatment. We also found that phorbol ester, known to activate PKC, blocked PCD in the sensitive populations. In a human synovial cell line sensitivity to cold shock-induced PCD is only observed after the cells reach confluence; in the logorithmic growth phase the cells are completely resistant (23). Interestingly, the logorithmic-phase cells become sensitive to PCD upon treatment with PKC antagonists, whereas phorbol esters inhibit PCD in their confluent counterparts. Therefore, proliferative and/or PKC status may dictate cellular sensitivity to PCD. If apoptosis contributes to the effects of glucocorticoids or chemotherapy in responsive patients with leukemia or other neoplastic disorders, it is certainly conceivable that the sensitivity of the transformed cells to apoptosis may affect whether a patient responds favorably. Further work will be required to identify the factors affecting the sensitivity of B-CLL and other neoplastic cells to PCD.

REFERENCES

1. **Wyllie, A. H., J. F. R. Kerr, and A. R. Currie.** 1980. Cell death: the significance of apoptosis. *Int. Rev. Cytol. 68:251.*
2. **Wyllie, A. H.** 1980. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature 284:555.*
3. **McConkey, D. J., P. Hartzell, P. Nicotera, and S. Orrenius.** 1989. Calcium-activated DNA fragmentation kills immature thymocytes. *FASEB J. 3:1843.*
4. **Cohen, J. J., and R. C. Duke.** 1984. Glucocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. *J. Immunol. 132:38.*
5. **Wyllie, A. H., R. G. Morris, A. L. Smith, and D. Dunlop.** 1984. Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. *J. Pathol. 142:67.*
6. **Smith, C. A., G. T. Williams, R. Kingston, E. J. Jenkinson, and J. J. T. Owen.** 1989. Antibodies to CD3/T-cell receptor complex induce death by apoptosis in immature T cells in thymic cultures. *Nature 337:181.*
7. **McConkey, D. J., P. Nicotera, P. Hartzell, G. Bellomo, A. Wyllie, and S. Orrenius.** 1989. Glucocorticoids activate a suicide process in thymocytes through an elevation of cytosolic Ca^{2+} concentration. *Arch. Biochem. Biophys. 269:365.*
8. **McConkey, D. J., P. Hartzell, S. K. Duddy, H. Håkansson, and S. Orrenius.** 1988. 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin kills immature thymocytes by Ca^{2+}-mediated endonuclease activation. *Science 242:256.*
9. **McConkey, D. J., P. Hartzell, J.-F. Amador-Perez, S. Orrenius, and M. Jondal.** 1989. Calcium-dependent killing of immature thymocytes by stimulation via the CD3/T cell receptor complex. *J. Immunol. 143:1801.*
10. **Shi, Y., B. M. Sahai, and D. R. Green.** 1989. Cyclosporin A inhibits activation-induced cell death in T-cell hybridomas and thymocytes. *Nature 339:625.*
11. **Ashwell, J. D., R. E. Cunningham, P. D. Noguchi, and D. Hernandez.** 1987. Cell growth cycle block of T cell hybridomas upon activation with antigen. *J. Exp. Med. 165:173.*
12. **Mercep, M., J. A. Bluestone, P. D. Noguchi, and J. D. Ashwell.** 1988. Inhibition of transformed T cell growth in vitro by monoclonal antibodies directed against distinct activating molecules. *J. Immunol. 140:324.*
13. **Boyd, A. W., and J. W. Schrader.** 1981. The regulation of growth and differentiation of a murine B cell lymphoma. II. The inhibition of WEHI 231 by anti-immunoglobulin antibodies. *J. Immunol. 126:2466.*
14. **Scott, D. W., J. Tuttle, D. Livnat, W. Haynes, J. P. Cogswell, and P. Keng.** 1985. Lymphoma models for B-cell activation. II. Growth inhibition by anti-μ of WEHI-231 and selection and properties of resistant mutants. *Cell. Immunol. 93:124.*
15. **Distelhorst, C. W.** 1988. Glucocorticosteroids induce DNA fragmentation in human lymphoid leukemia cells. *Blood 72:1305.*
16. **Aguilar-Santelises, M., J. F. Amador, H. Mellstedt, and M. Jondal.** 1989. Low IL-1 beta production in leucemic cells from progressive B cell chronic leukemia (B-CLL). *Leukemia Res. 13:937.*
17. **Burton, K.** 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J. 62:315.*
18. **Grynkiewicz, G., M. Poenie, and R. Y. Tsien.** 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem. 260:3440.*
19. **Gordon, J., H. Mellstedt, P. Åman, P. Biberfeld, M. Björkman, and G. Klein.** 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.*
20. **Jones, D. P., D. J. McConkey, P. Nicotera, and S. Orrenius.** 1989. Calcium-activated DNA fragmentation in rat liver nuclei. *J. Biol. Chem. 264:6398.*
21. **McConkey, D. J., P. Hartzell, M. Jondal, and S. Orrenius.** 1989. Inhibition of DNA fragmentation in thymocytes and isolated thymocyte nuclei by agents that stimulate protein kinase C. *J. Biol. Chem. 264:13399.*
22. **McConkey, D. J., P. Hartzell, S. C. Chow, S. Orrenius, and M. Jondal.** 1990. Interleukin 1 inhibits T cell receptor-mediated apoptosis in immature thymocytes. *J. Biol. Chem. 265:3009.*
23. **Perotti, M., F. Toddei, F. Mirabelli, M. Vairetti, G. Bellomo, D. J. McConkey, and S. Orrenius.** 1990. Calcium-dependent DNA fragmentation in human synovial cells exposed to cold shock. *FEBS Lett. 259:331.*
24. **Kumar, S., G. D. Baxter, P. J. Smith, L. Pemble, R. J. Collins, R. L. Prentice, and M. F. Lavin.** 1987. DNA fragmentation in childhood T-cell acute lymphoblastic leukaemia. *Mol. Biol. Med. 4:111.*
25. **Collins, R. J., L. A. Verschuer, B. V. Harmon, R. L. Prentice, J. H. Pope, and J. F. R. Kerr.** 1989. Spontaneous programmed death (apoptosis) of B-chronic lymphocytic leukemia cells following their culture *in vitro*. *Br. J. Haematol. 71:343.*
26. **Trauth, B. C., C. Klas, A. M. J. Peters, S. Matzku, P. Möller, W. Falk, K.-M. Debatin, and P. H. Krammer.** 1989. Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science 245:301.*
27. **Miller, R. A., D. G. Maloney, R. Warnke, and R. Levy.** 1982. Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. *N. Engl. J. Med. 306:517.*
28. **Meeker, T. C., J. Lowder, D. G. Maloney, R. A. Miller, K. Thielemans, R. Warnke, and R. Levy.** 1985. A clinical trial of anti-idiotype therapy for B cell malignancy. *Blood 65:1349.*
29. **Liu, Y. J., D. E. Joshua, G. T. Williams, C. A. Smith, J. Gordon, and I. C. M. MacLennan.** 1989. Mechanisms of antigen-driven selection in germinal centres. *Nature 342:929.*

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