Be13, a Human T-Leukemia Cell Line Highly Sensitive to Dexamethasone-induced Cytolysis

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INTRODUCTION

GC play a major role in the chemotherapeutic regimen of ALL. Administration of these hormones results in a decrease in the malignant lymphoid cell population in a proportion of the ALL patients (17). The GC effect in humans has been attributed to the inhibition of cell proliferation rather than to a direct lytic effect (6). This concept emerged from the findings that GC do not kill human thymocytes or peripheral blood lymphocytes but, rather, affect the mitogenic or allogeneic stimulation of peripheral blood lymphocytes (3, 4). In contrast, murine and rat thymocytes and lymphocytes are killed readily by these hormones (5, 7, 28). Humans have thus been designated a "GC-resistant" species, whereas mice or rats are "sensitive" species (6). In a series of studies, we have demonstrated recently several normal and malignant human lymphoid subsets which are lysed by the upper physiological and pharmacological concentrations of GC. These include human prothymocytes (13), activated T-cells (9, 15, 16), CLL cells (12, 14), and ALL cells from some of the patients tested (11, 14). Myeloid leukemia cells were found to be resistant to the GC-induced lysis (11, 14). In an attempt to understand the mechanism of this specific GC-induced lysis, the nonproliferating CLL cells (12, 14), and ALL cells from some of the patients included human prothymocytes (13), activated T-cells (9, 15, 16), CLL cells (12, 14), and ALL cells from some of the patients tested (11, 14). Myeloid leukemia cells were found to be resistant to the GC-induced lysis (11, 14). In an attempt to understand the mechanism of this specific GC-induced lysis, the nonproliferating CLL cells and immunoactivated T-cells were utilized for a model studying the lytic effect of glucocorticoids on the proliferating compartment of human leukemias.

ABSTRACT

A unique human T-leukemia cell line highly sensitive to dexamethasone-induced lysis is described. The cell line designated Be13 is killed readily within 24 hr by 10^{-9} M dexamethasone. No lysis is induced by nonglucocorticoid steroids. The lysis is mediated via specific cytoplasmic receptors and is efficiently blocked by the antagonist cortexolone. The inhibiting effect of actinomycin D and cycloheximide on the lytic process suggests the involvement of gene activation and destruction of the cells by an "autolytic protein." Kinetic studies imply that the lytic process is induced during a distinct phase of the cell cycle. Dexamethasone, however, does not cause an arrest in a distinct phase of the cell cycle. The Be13 cell is a unique human cell line killed directly by glucocorticoids, and it may serve as a suitable in vitro model for studying the lytic effect of glucocorticoids on the proliferating compartment of human leukemias.

MATERIALS AND METHODS

Cell Lines. The Be13 cell line was derived from the bone marrow cells of an 11-year-old patient with T-ALL in relapse (10). Other cell lines studied were the T-cell line HD-Mar, derived from a patient with Hodgkin’s lymphoma (2), the B-cell line Daudi, derived from a Burkitt’s lymphoma patient (20), and myeloid cell line K562, originally grown from a chronic myeloid leukemia patient in blast crisis (22). All cell lines were grown at 37°C in suspension culture by using the nutrient medium RPMI 1640 (Grand Island Biological Co., Grand Island, NY) supplemented with 10% (v/v) heat-inactivated fetal calf serum.

Surface Markers. E-receptor, Fc-receptor, T-cell antigen, Smig, and Ia antigen detection were performed according to previously described, routine methods (13).

In Vitro GC-induced Cytolysis. The test was performed as described previously (11-16). Briefly, the cells were incubated with various concentrations of steroids for 24 hr at 37°C in a 5% CO2 humidified atmosphere. All steroids used were purchased from Sigma Chemical Co. (St. Louis, MO). The percentage of cytosis was calculated by means of the formula

\[
\frac{(a - b)}{a} \times 100
\]

where a is the concentration of viable cells in control wells and b is the concentration of viable cells in wells containing the steroid. Viability was assessed by trypan blue exclusion test. The dye penetrates only into dead cells.

Measurement of Total Cellular Binding and Nuclear Translocation of Dexamethasone (34, 32). Two ml of 50 × 10^6 cells/ml were incubated with 2 μCi of 7 × 10^{-9} μ M [3H]dexamethasone (New England Nuclear; specific activity, 27 Ci/mmol), for 1 hr at 0°C or 37°C. High-affinity [3H]-dexamethasone binding was measured by addition of 0.2-ml aliquots of cells to 10 ml of medium (50-fold dilution) and incubation of the diluted...
Specific Dexamethasone-induced Lysis of Be13 Cells. More than 90% of the Be13 cells incubated with 10^{-8} to 10^{-5} M dexamethasone were lysed in a 24-hr period (Chart 1). Lowering the concentration of dexamethasone to 10^{-9} M resulted in a 60% lysis of the cells. Incubation of the cells for 48 hr with 10^{-4} M dexamethasone induced the lysis of 99% of the Be13 cells (not shown). The other GC hormone tested, cortisol, was less effective, however, causing 65% lysis at 10^{-5} M and 40% lysis at 10^{-6} M. Sex hormones such as estradiol, progesterone, or 3H-thymidine incorporation in Be13 cells. Aliquots of 1 ml containing 10^6 Be13 cells were incubated for 1 hr at 37° in presence of 1 mCi of [3H]thymidine (Nuclear Research Center, Negev, Israel). Thereafter, the cells were harvested on glass fiber filters and precipitated with cold 5% trichloroacetic acid. The filters were dried, and radioactivity was measured in Tricarb scintillation counter (Packard).

Involvement of Specific GC Receptors in the Lytic Process. Steroid action in mammalian cells is mediated via the interaction of the rapidly penetrating steroid molecules with specific cytoplasmic receptors. The formation of steroid-receptor complexes in the course of the GC-induced lysis of Be13 cells was studied by the use of: (a) specific GC antagonists; and (b) 3H-labeled dexamethasone. A 100-fold excess of cortisol, a potent antagonist for receptor binding which cannot elicit nuclear events (31), partially inhibited the lytic effect of 10^{-7} M dexamethasone (Chart 3). Complete inhibition was seen when 10^{-8} M or 10^{-9} M dexamethasone was used. Similarly, complete inhibition of lysis induced by 10^{-6} M cortisol was achieved by 10^{-4} M cortexolone (Table 1). The sex hormones estradiol and testosterone, which are partial antagonists (1), exhibited effective inhibition at 10^{-7} M concentration only with the very low concentration of 10^{-9} M dexamethasone. No inhibition effect was elicited by these sex hormones on the cytolytic activity of 10^{-8} M dexamethasone. Additional evidence for the presence of specific cytoplasmic receptors to GC was obtained by the binding of [3H]dexamethasone (Table 1). The amount of the bound steroids corresponds to about 10,000 receptor sites/cell. While GC-receptor interac-
DAYS OF INCUBATION

Chart 2. In vitro growth of various human cell lines in presence of 10^-6 M dexamethasone. O, Bel3 cells; G, HD-Mar cells; Δ, Daudi cells; V, K562 cells.

Number of viable cells/ml in all cell lines except Bel3 did not differ in control cultures from that observed in cultures containing the hormone. Growth curve of Bel3 control cultures is the same as that of Daudi cells.

Glucocorticoid Antagonist % of Lysis 5 7 9

dex 10^-7 M cortexolone 10^-5 M

dex 10^-6 M cortexolone 10^-5 M

dex 10^-5 M cortexolone 10^-5 M
dex 10^-5 M testosterone 10^-5 M
dex 10^-5 M estradiol 10^-5 M
cortisol 10^-6 M
cortisol 10^-6 M cortexolone 10^-5 M

Chart 3. Inhibition of GC-induced lysis of Bel3 cells by various antagonists. dex., dexamethasone.

tion is a prerequisite for induction of lysis, it is not sufficient for ultimate lysis. Thus, the binding of [3H]dexamethasone to the resistant cell lines was of a level similar to that of the sensitive Bel3 cells. Furthermore, no significant difference in the proportion of nuclear translocation of the bound steroid was noted in the different cell lines. In all cell lines tested, 70 to 80% of the receptor-bound [3H]dexamethasone was translocated to the nucleus during incubation of cells in 37° but not at 4° (Table 1).

Further analysis of the dexamethasone binding curve in Bel3 cells demonstrates a high-affinity interaction between the hormone and the cytoplasmic receptors (Chart 4A). Scatchard analysis of the binding data yielded a straight line with a slope representing a Kd of 5.55 nm. The X-intercept provided an estimate of 6500 receptors/cell, which is lower than that found in the above method, probably due to the difference in methodology. The binding curve of cortisol (Chart 4B) demonstrated a much lower affinity to the cytoplasmic receptors than that displayed by dexamethasone. The Kd for cortisol was found to be 2.73 nm using the first 5 points of the binding curve. The actual Kd is even lower, since the binding curve did not reach a saturation in the cortisol concentration used. Accordingly, at 10 nm, GC concentration, dexamethasone occupied 6500 receptors, whereas cortisol bound to only 300 receptor molecules.

Kinetics of the Lytic Process. Be13 cells incubated with 10^-7 M dexamethasone for 24 hr were almost entirely lysed (>90%). This lysis could not be observed in the light microscope before 16 hr of incubation and, at 20 hr of incubation, only 60 to 80% of cells were killed (Chart 5). The presence of the hormone in the medium for the entire incubation period was essential for lysis. When dexamethasone was removed after 8 hr, no lysis was observed at the end of the 24- or 48-hr period. Exposure of the cells for 12 hr to 10^-7 M dexamethasone, however, resulted in 20% lysis at the end of an additional 12-hr incubation and 100% lysis following 20 hr of incubation in the absence of the hormone (Chart 5). Incubation for 16 hr with dexamethasone resulted in complete lysis of the cells only after an additional 16 hr of incubation without the hormone.

These findings suggest that the lytic process is induced during specific stages of the cell cycle. A substantial proportion of the cells must pass this specific stage before a measurable lytic effect is seen. Dexamethasone does not, however, cause an arrest in the cell cycle of the nonlysed population of cells. This is demonstrated clearly by the findings that DNA synthesis of the 10^6 viable cells did not vary following 24 hr of incubation with 10^-7 M dexamethasone (Chart 6). Furthermore, while only 5% of...
A Human T-Cell Line Sensitive to Dexamethasone Lysis

Chart 5. Cytolysis of Be13 cells following 12-hr (■) and 16-hr (□) preincubation with 10^{-7} M dexamethasone. Time 0 represents removal of dexamethasone from cultures. ○ control cultures in which the hormone is present during the incubation period.

Chart 6. Be13 cell cycle parameters in the presence of 10^{-7} M dexamethasone. ○, % of Be13 cell lysis; △, % of mitotic figures among viable cells; □, [3H]thymidine incorporation to 10^6 alive Be13 cells. For assessment of [3H]thymidine incorporation at 16, 20, and 24 hr, cell suspensions were brought to a concentration of 10^6 viable cells/ml.

Ultrastructural Changes in the Course of Lysis. Analysis by scanning electron microscope showed that no changes occurred in the cells incubated with dexamethasone for 8 hr. At 16 hr, a loss of microvilli resulting in a smoothening of the cell surface was seen (Figs. 1 and 2). This was followed by the appearance of pores in the cell membrane. Observations in a transmission electron microscope indicated disruption in polysomes and homogenous distribution of ribosomes, as one of the first morphological alterations in the affected cell cytoplasm. This occurred at 16 hr (Figs. 3 to 6). The loss of microvilli was a concurrent event. Pyknosis of the nucleus was noted only in advanced stages of the lytic process, together with the disruption of the cell membrane and destruction of cytoplasmic organization (Fig. 7).

Transcriptional and Translational Events in the Course of Lysis. The model of steroid action includes the synthesis of specific mRNA and protein molecules for the manifestation of the biological effect of the hormone (8). In Be13 cells, inhibitors of RNA and protein synthesis reduced the lytic effect of dexamethasone. When the RNA inhibitor actinomycin D was added at a concentration of 0.1 μg/ml, complete inhibition of lysis induced by 10^{-7} and 10^{-6} M dexamethasone was observed (Chart 7). A partial inhibition of the lysis occurred with 10^{-5} and 10^{-4} M of the hormone. The protein synthesis inhibitor cycloheximide (1 μg/ml) exhibited a more potent inhibitory effect and abolished the lysis induced by dexamethasone, even at the high concentration of 10^{-5} M.

DISCUSSION

The Be13 cell line, originating from a T-ALL patient, was found to be highly sensitive to GC-induced lysis and, thus, it could be used to study the lytic phenomenon in proliferating malignant lymphoid cells. In contrast to freshly isolated leukemic cells, in which cortisol was as effective as was dexamethasone in inducing lysis (12), a significant difference in the killing potency of these 2 hormones was observed when Be13 cells were the target cells. Dexamethasone at a concentration of 10^{-6} M was sufficient to induce 60 to 70% lysis during 24 hr as compared to 10^{-5} M cortisol. This difference seems to be due to a higher affinity of the dexamethasone for the cytoplasmic receptors. The different affinities of these 2 GC hormones to the cytoplasmic receptors of the Be13 cells was demonstrated clearly in the binding curve experiments with the radiolabeled hormones and by the K_d values obtained. The competitive inhibition effect exerted by the antagonist cortexolone further indicated the formation of steroid-receptor complexes. A 1000-fold increase in the antagonist concentration competed with 10^{-8} M dexamethasone to abolish the lytic effect. A 10-fold increase was sufficient, however, to block the lytic effect of 10^{-6} M cortisol, further suggesting that dexamethasone does, indeed, bind with a higher affinity to the cytoplasmic receptor.

Both actinomycin D and cycloheximide inhibited the lysis of Be13 cells by dexamethasone, suggesting that gene activation is involved in the lytic action of GC. In line with the general model of steroid action, the killing of Be13 cells involves the translocation of the dexamethasone-receptor complex into the nucleus, where it apparently activates a gene(s) to synthesize a specific mRNA and subsequent "autolytic" protein. The presence of this...
protein is only hypothetical, since it has not as yet been isolated and identified. The antagonistic effect of the metabolic inhibitors raises the need, however, for a critical evaluation of the clinical benefit of the administration of drugs with such an activity, concurrently with GC in the course of chemotherapeutic regimens.

When analyzed by electron microscopy, the morphological features of the lytic process were found to resemble those of the nonproliferating CLL cells (11). Loss of membrane microvilli and the disappearance of polysomal organization preceded the appearance of membranal pores and nuclear destruction. In contrast, the kinetics of cell lysis was found to differ greatly in the 2 cell types. Whereas CLL death could be detected within 6 hr of incubation with GC, only at 16 to 18 hr could lysis be observed in the Be13 cell line. Exposure of CLL cells to the hormone for a short period of 30 min was sufficient to evoke an irreversible lytic process (12); in the Be13 cells, incubation of at least 12 hr was required to induce cell lysis following an additional 36 hr in culture.

These results suggest that the induction of lysis in a proliferating cell is dependent on a specific stage of the cell growth cycle. It is possible that the genes required for activation by the steroid-receptor complex are "accessible" only at a specific phase of the cell cycle. CLL cells, which are all at the same phase, require only a short exposure to the steroid for induction of the lytic process. Studies on synchronized Be13 cells are presently being conducted to clarify this point.

The sensitivity of a particular malignant cell to GC appears to be dependent upon the state of differentiation in which the cell is arrested. The Be13 cell, arrested at the prothymocyte stage of T-cell differentiation (10), is highly sensitive to GC-induced lysis. In contrast, the HD-Mar cell line, which reflects the more mature cortical thymocyte differentiation state, is resistant to a concentration as high as 10⁻⁶ M dexamethasone. The B-cell line Daudi and the myeloid cell line K562 are similarly resistant. These findings correlate well with our previous studies on normal human lymphoid cells (12–16). Human prothymocytes and activated T-cells were found to be sensitive to GC-induced lysis, while the cortical thymocytes, peripheral T-cells, B-cells, and myeloid cells were resistant. Thus, the transformation event seems not to change the degree of sensitivity of the cell to steroid-induced lysis. Establishment of additional human GC sensitive cell lines is needed for confirmation. It should be noted that dexamethasone was found to bind equally well to the resistant and sensitive cell lines. Furthermore, translocation to the nucleus is similar in all cell lines. This is in agreement with our previous findings (9) and those of other investigators (19, 33), demonstrating no significant difference in these 2 parameters in GC-resistant and -sensitive cells. It should be noted, however, that other studies have shown that, in certain lymphoid cell lines and some freshly isolated leukemic cells, the GC effect does relate to the number of receptors per cell (21, 27). Various, not fully understood, cellular factors thus seem to be involved in rendering a cell sensitive to GC lytic action. Analysis of the variations in cytoplasmic receptors (23, 29) and identification of the relevant gene(s) in relation to specific differentiation states will be helpful in understanding the cause of cell resistance to GC therapy.

Thus far, Be13 is the only known human cell line which is directly lysed by GC. Harmon et al. (18) have reported on a human T-cell line, CEM-7, which is arrested in the G1 phase following exposure to 10⁻⁶ M dexamethasone for 2 to 3 days. At Day 4 to 5, cells become pyknotic and are lysed. The death of the CEM-7 cells seems to be secondary to the arrest in cell cycle. Thus, the effect of dexamethasone on these cells was most prominent in reducing the plating efficiency of the cell line, analyzed after 10 to 14 days of incubation with the hormone. No plating efficiency changes could be tested with the Be13 cells, since they were unable to form colonies in soft agar; however, the Be13 cells, unlike CEM-7 cells, are lysed already within 16 to 20 hr by a direct mechanism which seems to involve specific gene activation. Furthermore, the cell cycle does not seem to be affected by the dexamethasone, as indicated by the unaltered proportion of Be13 cells in S- and M-phases within the small viable cell population remaining after 24 hr of exposure to the hormone. Nevertheless, both studies indicate that proliferating leukemic cells must be exposed for relatively prolonged periods to GC in order to elicit a response. This suggests that effective therapeutic response is dependent on a continuous administration of the steroids. In view of the short half-life of steroids in circulation, administration of GC in pulses may result in the selection of relatively resistant clones of proliferating leukemic cells. At present, we are attempting to isolate resistant clones of Be13 cells in order to investigate the mechanisms enabling GC-sensitive human leukemias to escape total eradication by steroid therapy.

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REFERENCES


Fig. 1. Be13 cells in control cultures; note the elongated microvilli. × 6000. Figs. 1 and 2, scanning electron microscope of Be13 cells in the course of 10^{-7} M dexamethasone-induced lysis.

Fig. 2. Be13 cells incubated for 16 hr with dexamethasone. One of the cells displays normal features and the second has lost microvilli, whereas the third cell is dead, as indicated by the porous cell membrane. × 5500.

Fig. 3. Be13 cells in control cultures; note the convoluted nucleus characteristic to thymic lymphocytes and T-leukemia cells. × 12,000. Figs. 3 to 8, transmission electron microscope of Be13 cells in the course of 10^{-7} M dexamethasone-induced lysis.

Fig. 4. Portion of a Be13 cell; note the distinct polysomes comprised of 4 to 6 ribosomes. × 45,000.

Fig. 5. Cells incubated for 16 hr with dexamethasone; note the loss of microvilli. × 9500.

Fig. 6. A portion of a Be13 cell following 16 hr of incubation with dexamethasone; note the absence of polysomes and the homogenous dispersion of free ribosomes. × 45,000.

Fig. 7. Be13 cells following 20 hr of incubation with dexamethasone; note the disintegration of membranal structure and of the intranuclear heterochromatin organization. × 7500.

Fig. 8. Dividing cells containing distinct chromosomes within the culture incubated for 20 hr with the hormone. × 6500.
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