J. Steroid Biochem. Molec. Biol. Vol. 42, No. 1, pp. 1–9, 1992
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0960-0760/92 $5.00 + 0.00
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GLUCOCORTICOID, INTERLEUKIN-2, AND PROSTAGLANDIN INTERACTIONS IN A CLONAL HUMAN LEUKEMIC T-CELL LINE

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(Received 17 July 1991)

Summary—We have studied the growth effects of conditioned media, interleukin-2 and PGE prostaglandin analogs on the glucocorticoid-sensitive human leukemic T-cell clone, CEM-C7. After 4 days, the glucocorticoid dexamethasone at ~10 nM kills 50% of CEM-C7 cells. To test the hypothesis that glucocorticoid-mediated lymphocytolysis was due to suppression of lymphokine expression only, we attempted to protect CEM-C7 cells from lysis by provision of lymphokine(s). Conditioned media from interleukin-2 secreting Jurkat T-cells as well as the glucocorticoid-insensitive, but receptor positive clone, CEM-C1, failed to prevent lymphocytolysis; exogenous interleukin-2 also did not provide protection. There were complex, biphasic interactions between dexamethasone and the synthetic PGEs, enisoprost and enisoprost free acid. Low doses of enisoprost alone (0.01 to 1 μg/ml) stimulated growth, and in combinations completely reversed the growth inhibitory effects of 10 nM dexamethasone. Higher concentrations of enisoprost were inherently lethal and were additive to the steroid effect. Thus the glucocorticoid-induced lymphocytolysis in this human leukemic T-cell line may be modified biphasically by PGE prostaglandins, depending on their concentration. However, interleukin-2 or components in the conditioned media assayed had no effect in ameliorating the lethal response to glucocorticoid.

INTRODUCTION

Glucocorticoids, interleukins and prostaglandins all have profound and interwoven effects on lymphoid cells. Glucocorticoids have long been known for their growth suppressive, even cytolytic, effects on certain lymphoid cells, particularly immature T-cells. These effects have been utilized to treat various leukemias and lymphomas. Treatment of sensitive leukemic cells with glucocorticoids leads to inhibition of cell proliferation and cell lysis. While these events require functional glucocorticoid receptors, the subsequent pathway(s) of action are at present unclear. Specifically, it is not known whether or how interleukins and/or prostaglandins may participate in or modify the steroid effects.

One potential mechanism of glucocorticoid-induced lymphoid cell death is inhibition of lymphokine production. Among the lymphokines, interleukin-2 (IL-2) has been shown to be essential for normal T lymphocyte proliferation by initiating the transition from G1 to S phase of the cell cycle [1, 2]. Glucocorticoids reduce the production of IL-2 and its mRNA in murine cytolytic T lymphocytes, bovine lymphocytes, and human peripheral blood lymphocytes [3–5]. In 1977, Smith *et al.* [6] first reported that thymidine and uridine uptake in unstimulated and stimulated human lymphocytes were suppressed by glucocorticoids, but that there was no alteration of viable cell numbers after 19 h. Later the same laboratory demonstrated that in IL-2 dependent murine cytolytic T-cell lines, the dexamethasone (Dex) inhibition of mitogen-activated lymphocyte proliferation as assayed by [^{3}H]thymidine incorporation was overcome by conditioned medium containing IL-2 [3]. In mouse spleen cell cultures, Larsson [7] concluded that the "general suppressive effects" of Dex on the proliferation of all T lymphocyte clones occurred only via the inhibition of IL-2 production.

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Abbreviations: IL, interleukin; PG, prostaglandin; Dex, dexamethasone; PHA, phytohemagglutinin; PMA, phorbol myristate acetate; CTLL, cytotoxic T-cell line; E, enisoprost; EFA, enisoprost free acid; PBMC, peripheral blood mononuclear cells.

Whether this generalization, that all lympho-inhibitory effects of glucocorticoids are mediated by suppression of essential lymphokine production, carries over to the inhibitory/lethal effects of glucocorticoids on leukemic cells is not clear. Furthermore, recent investigations indicate that in addition to IL-2, other factors such as IL-2 receptors, IL-4, glucocorticoid receptor density, IL-3, granulocyte-macrophage colony stimulating factor, and certain eicosanoids may be involved in modulating the steroid antiproliferative responses of lymphoid cells [8–13].

Prostaglandins are also known to be potent mediators of the immune response, and various members of this eicosanoid subcategory can stimulate or suppress growth and function of T-cells [14–16]. In sufficient amounts both endogenous and synthetic prostaglandins of the PGE type are immunosuppressive [16]. There are elevated levels of plasma PGE in cancer patients [17], and it has been proposed that immune suppression by solid tumors is due to their overproduction of certain prostaglandins, especially PGEs [18, 19]. While many cells synthesize prostaglandins, normal lymphocytes apparently do not [20]. Thus prostaglandins affect T-cells, but are not made by them. Various lines of research have found that glucocorticoids inhibit prostaglandin biosynthesis [21–25], and in combination with PGEs these steroids also reduce normal lymphocyte immunological responsiveness [26]. Thus, it is of obvious importance to determine the interactive effects of glucocorticoids and prostaglandins on leukemic cells.

The T-cell derived human acute lymphoblastic leukemia cell line CEM provides a well-defined, glucocorticoid-sensitive system in which to study the mechanisms of glucocorticoid action and resistance [27]. CEM-C7 cells, cloned from the CCRF-CEM line, are 90% T4/CD4 + [28]. They are arrested in G1 phase and eventually lysed by glucocorticoids at concentrations that saturate the glucocorticoid receptor [29–31]. In addition, several glucocorticoid-resistant clones of various phenotypes derived from either CCRF-CEM or CEM-C7 have been isolated. CEM-C7 cells do not require exogenous IL-2 for their growth [28], and are lysed by glucocorticoids. However in principle, IL-2 could be made by the cells, functioning as an autocrine or paracrine growth factor. In that case, its repression by the glucocorticoid might result in growth cessation and cell death. Resistant clones might be those in which this negative control by glucocorticoid was lost. In our investigation of this possibility several questions pertain: (1) do any of the resistant CEM clones constitutively produce IL-2 or other cytokines/factors, thus conferring glucocorticoid resistance? (2) Would exogenous IL-2 prevent the glucocorticoid effects on the sensitive clone? (3) Are other endogenous factors involved in resistance or sensitivity to the steroids? (4) Is the direct effect of glucocorticoids on these cells mediated or modified by prostaglandins? By isolating the leukemic T-cell in tissue culture, we have shown that the lethal effects of glucocorticoids can be direct on such cells, and not mediated indirectly by altering production of other cells' growth factors, upon which the leukemic cells depend. However, it is well known that various tumors can make steroid-mediated growth factors [32]. Because of that, and since normal lymphocytes make various hormones and receptors [33], it is possible that CEM cells drive their own growth via paracrine production of critical growth factors. We therefore tested the hypothesis, and its corollaries, that glucocorticoids induce CEM cell death by altering the production of vital paracrine growth factors made by these leukemic cells.

Regardless of whether steroids' direct anti-leukemic action is by altering paracrine factor production in the actual leukemic cell, treatment with the hormones *in vivo* inevitably leads to alteration in lymphokine and prostaglandin production by normal cells. The extent to which such altered factors affect the steroids' attack on leukemic cells is unknown. We therefore also examined a second hypothesis, that glucocorticoids' lethal effects on leukemic cells can be modified directly in malignant cells by the influence of other compounds known to affect lymphoid cells.

In summary, this study tests the hypothesis that suppression of IL-2 or other factor(s) produced by glucocorticoid receptor positive T-cells is the primary mechanism by which lymphocytolysis occurs in these leukemic cells and investigates a possible role of prostaglandins in steroid-induced lysis.

EXPERIMENTAL

Materials

RPMI 1640 culture medium was obtained from Fisher (Houston, TX); fetal bovine serum

from Hazelton Biologics (Lenexa, KS); phytohemagglutinin (PHA) from Wellcome Research (Beckenham, England); phorbol myristate acetate (PMA), rat splenocyte IL-2 (ammonium sulfate precipitates from conditioned medium) and dexamethasone (9α-fluoro-16α-methyl-11β,17α,21-trihydroxy-pregna-1,4-diene-3,20-dione) from Sigma (St Louis, MO); dialysis tubing from BRL (Bethesda, MD); and trypan blue dye from GIBCO (Grand Island, NY). Enisoprost (methyl-11α,16-dihydroxy-16-methyl-9-oxoprosta-4Z,13E-dien-1-oate) and enisoprost free acid were a gift from G.D. Searle Research and Development (Skokie, IL). Tritiated thymidine (26 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). CoStar (Cambridge, MA) supplied the 6-well cluster as well as the Transwell 6-well culture dishes. The Jurkat cells were a gift from Dr Courtney Townsend, Department of Surgery, The University of Texas Medical Branch (Galveston, TX).

Cell culture

Human leukemic T-cells: CEM-C7, CEM-C1, and Jurkat were cultured in RPMI 1640 containing 5–10% heat inactivated fetal bovine serum at 37°C in humidified 95% air–5% CO_2 incubators. Cultures were maintained in log growth phase between experiments. Initial cultures for experiments were approx. $1–2 \times 10^5$ cells/ml with all assays in duplicate or triplicate in 6-well cluster culture dishes containing 1–2 ml cell culture/well. Stock aliquots of Dex and enisoprost or enisoprost free acid were prepared in ethanol; IL-2 was dissolved with RPMI 1640 medium. Alcohol concentrations in assays did not exceed 1% by volume. The appropriate carrier was added to control cultures in all experiments and had no effect on cell proliferation.

Cell number, viability and thymidine uptake

Growth experiments were conducted in 6-well cluster dishes with cells distributed at twice their final concentration to all wells, immediately followed by addition of drug(s) at 2 × concentration(s) in an equal volume of culture medium, followed by thorough mixing. A Model Z_f Coulter Counter (Hialeah, FL) was used to determine initial cell density. Settings of 2 for 1/amplification, 10 for threshold, and 1 for 1/aperture were found empirically to provide the best estimate of viable cells. Since Coulter Counter evaluation of cell density does not entirely distinguish viable from dead CEM cells, manual cell counts by hemacytometer using a trypan blue dye exclusion viability assay (2 sample aliquots of 100 μl each + 40 μl of 0.4% trypan blue dye or 100 μl sample aliquot + 100 μl diluent + 100 μl dye) were conducted on day 3 and/or day 4 after subculturing. Viable CEM cells have the ability to exclude this dye under our test conditions for at least 15 min, and all counts were made within 5 min. We have observed previously in CEM cells that there is good correlation with the trypan blue dye exclusion test and number of viable cells as measured by clonogenicity [29].

Tritiated thymidine uptake was determined by adding 5 μl [^{3}H]thymidine to 1 ml of cell culture and aliquoting four 200 μl samples into a 96-well plate. After 3 h incubation, the cells were harvested on a Cambridge Technology PHD Cell Harvester (Watertown, MA) using glass fiber filters. After drying the filter disks overnight at 37°C in scintillation vials, aqueous cocktail (3 ml) was added, the sample thoroughly mixed, the disintegrations/min determined on a Beckman Scintillation Counter Model LS5801 (Houston, TX). Final evaluation of viable cell number and thymidine uptake was conducted 4 days after treatment.

Conditioned media

CEM-C7 or CEM-C1 cells were maintained in log growth for about 4 days, until cell density reached approx. 1×10^6 cells/ml, at which time the cells were pelleted at 1000 rpm for 10 min, and the supernatant conditioned media removed, filtered through 0.2 μm pore sterile filters and stored at 4°C. CEM-C1 conditioned media derivatives were prepared as follows: (1) 10 ml conditioned medium was dialyzed for 18 h at 4°C against 1 l RPMI 1640 medium without serum in 12,000–14,000 molecular weight pore size dialysis tubing. The medium inside the tubing (retentate) was adjusted to its original volume and used for culturing CEM-C7 cells; (2) by lyophilizing 10 ml of conditioned medium, reconstituting in 10 ml of fresh RPMI 1640 with serum and dialyzing as above; (3 and 4) by dialyzing as in protocol 1 above, lyophilizing the retentate as well as the medium outside the tubing (dialysate) and reconstituting both in 2 ml fresh RPMI 1640 with serum. All samples were sterilized by filtration before use.

Jurkat cell cultures at 1×10^6 cells/ml were stimulated with 1% by weight PHA and 10 ng/ml PMA; the conditioned medium was collected after 24 h and stored at 4°C [34].

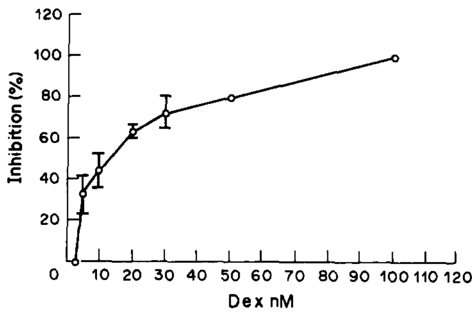

Fig. 1. Percent inhibition (i.e. loss) of viable CEM-C7 cells after 4 days of treatment with increasing concentrations of Dex. Bars indicate standard deviation, $n = 11$ (average); no apparent bars indicate standard deviation <symbol. Viable cells were determined by trypan blue dye exclusion assay.

Jurkat cell conditioned medium produced for this study was tested for IL-2 activity by Dr Gary Klimpel, Microbiology Department, The University of Texas Medical Branch (Galveston, TX). Using the ability of the medium to sustain the growth of an IL-2 dependent mouse cytotoxic T-cell line (CTLL), our Jurkat conditioned medium contained approx. 100 units IL-2/ml.

In all assays utilizing conditioned media 1×10^5 CEM-C7 cells/ml were suspended in 2 ml conditioned medium plus 1 ml fresh RPMI 1640 medium with 2 wells for controls (containing ethanol as vehicle) and 2 wells treated with 10 nM Dex. On day 4 viable cell density was determined by trypan blue dye exclusion.

Dual cultured cell conditioned media

The bottom well of a Transwell 6-well culture dish contained 2.6 ml of one type of cell at 2×10^5 cells/ml which was separated from the top well by a 0.4 μm pore polycarbonate membrane to permit exchange of media. The top well contained 1.5 ml of second cell type at 3×10^5 cells/ml. Three wells served as controls (<1% ethanol added as vehicle) and 3 wells were treated with 10 nM Dex. On day 4 viable cell density of both cell types was determined by trypan blue dye exclusion.

RESULTS AND DISCUSSION

Growth inhibition by dexamethasone

CEM-C7 cells provide a suitable model to study glucocorticoid sensitivity. By 4 days' treatment, inhibition of cell growth as recorded by reduced numbers of viable cells occurs at concentrations of Dex >3 nM, with about 50% cell lysis at 10 nM and virtually 100% cell death by 100 nM (Fig. 1). In addition to the well-characterized, glucocorticoid-sensitive clone, CEM-C7, we have described a closely related CEM clone, C1, which has functional glucocorticoid receptors but nevertheless is lysis resistant [35]. Dex inhibits the growth of CEM-C7 cells, but not CEM-C1 cells, as illustrated in Fig. 2. There is no difference in the growth of viable cells between the CEM-C1 control and glucocorticoid-treated cultures, whereas 10 nM Dex treatment of CEM-C7 cultures reduces viable cells to about 50% of controls after 4 days.

If the proliferative as well as cytolytic effects of corticosteroids on T-derived lymphocytes are via modifying endogenous lymphokine activities, then a glucocorticoid-sensitive clone such as CEM-C7 might be rendered resistant by providing the necessary lymphokine(s) during culturing. Previous studies have indicated that certain T lymphocyte cell lines produce lymphokines-enriched culture supernatants [34, 36–39]. It has been reported that the uncloned CEM line does not make IL-2 [40], but of course our specific clone CEM-C7 and its progeny might do so and not have been detected in the mass of cells comprising the uncloned CEM line. In adult T-cell leukemia, a single T lymphocyte can produce multiple lymphokines, with different combinations of lymphokines being produced by closely related clones [41]. Thus a resistant clone such as CEM-C1 could

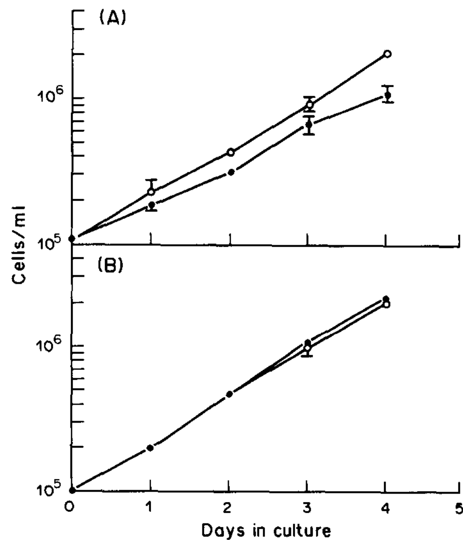

Fig. 2. 10 nM Dex inhibits growth of CEM-C7 cells (A) but not CEM-C1 cells (B). Control cultures had added ethanol vehicle only; bars indicate standard deviation; no apparent bars indicate standard deviation <symbol. Viable cells were determined by trypan blue dye exclusion assay from duplicate values of triplicate samples at times shown.

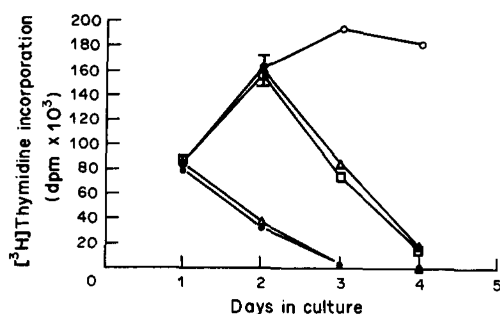

Fig. 3. Tritiated thymidine uptake as determined by disintegrations per minute of vehicle-treated control cultures of CEM-C7 (○), CEM-C7 cultures with 10 nM Dex added at time 0 (●), CEM-C7 cultures with 10 nM Dex added after 24 h (△), CEM-C7 cultures with 1 U/ml IL-2 and 10 nM Dex added at time 0 (▲), and CEM-C7 cultures with 1 U IL-2 added at time 0 and 10 nM Dex added after 24 h (□). Bars indicate standard deviation, $n = 4$; no apparent bars indicate standard deviation <symbol.

provide CEM-C7 cells the necessary factor(s) to overcome cytolysis by glucocorticoids. However, it has been reported that most CEM clones are killed directly by glucocorticoids and that adding interleukin-enriched medium to the culture did not block the lysis effect [42]. We have now more thoroughly studied the possibility that factors made by resistant T-cells could confer resistance to glucocorticoid-induced lysis.

Response to conditioned media

Utilizing the CEM-C7 conditioned medium (see Experimental), we first determined that untreated CEM-C7 cultures did not themselves produce a substance(s) which would provide protection against the cytolytic effect of glucocorticoids at either the pharmacologic concentration of 1 μM Dex or the roughly half receptor-saturating concentration of 10 nM Dex (data not shown). This partially lethal glucocorticoid concentration was chosen so as to provide maximal sensitivity for detection of rescue from steroid-induced growth inhibition by factor(s) in conditioned media.

The glucocorticoid-insensitive clone, CEM-C1, might be a more likely candidate to provide such factor(s) since its resistance appears not to be due to faulty glucocorticoid receptors, but to some other unidentified mechanism [35]. Several different assays utilizing CEM-C1 conditioned media prepared in various ways were run to detect a putative Dex-lysis protective factor(s): (1) CEM-C1 conditioned medium was added directly to CEM-C7 cultures. (2) CEM-C1 conditioned media prepared by four different dialysis procedures (see Experimental) was added to CEM-C7 cultures. In every assay 2 ml of these conditioned media were diluted with 1 ml fresh RPMI 1640 medium with serum and tested for their ability to protect CEM-C7 cells from 10 nM Dex over several days. (3) Challenged with 10 nM Dex treatment CEM-C7 cells (bottom well) were co-cultured with CEM-C1 cells (top well) in dual wells to provide a continuous source of possible growth factors. None of these approaches using CEM-C7 or CEM-C1 cell supernatants conferred protection to CEM-C7 cells treated with 10 nM Dex (data not shown).

Clonal lines derived from CCRF-CEM have been reported to be poor lymphokine producers [43]. Santoli *et al.* [44] found uncloned CCRF-CEM to be one of the most active producers of a lymphokine capable of *inhibiting* proliferation. On the other hand, Jurkat cells make few inhibitory substances and have been shown to produce between 100–200 times the amount of IL-2 normally generated by normal human blood lymphocytes [34]. Concentrations

Table 1. Glucocorticoid-induced growth inhibition of CEM-C7 cells uneffected by exogenous IL-2 or IL-2 conditioned media: four days treatment

Treatment	% Inhibition	%[³H]thymidine[b]
(A) Exogenous IL-2		
10 nM Dex	66 ± 9.2	
+5 U/ml IL-2	97 ± 3.5	
+25 U/ml IL-2	94 ± 2.7	
+125 U/ml IL-2	100 ± 0	
+325 U/ml IL-2	100 ± 0	
(B) Conditioned medium ± exogenous IL-2		
10 nM Dex	90 ± 0.7	16 ± 3.4
+1 U/ml IL-2	86 ± 13.4	21 ± 7.8
+1 U/ml IL-2 in Jurkat conditioned medium	85 ± 0.7	12 ± 1.2

[a]% Inhibition of cell densities of treated cell cultures vs control cell cultures expressed as the percentage below 100% growth with standard deviation.
[b]% Tritiated thymidine uptake of treated cell cultures vs control cultures.
[c]The co-culturing of CEM-C7 cells with human lymphocytes resulted in slower cell doubling times for control cultures and may account for the unusually high inhibitory effect of 10 nM Dex.

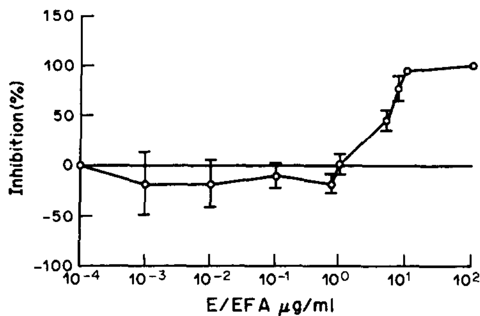

Fig. 4. Percent inhibition of viable CEM-C7 cells after 4 days treatment with increasing concentrations of E or EFA with standard deviation bars, $n = 11$ (average). The response of E and EFA was qualitatively the same: data represents average of E + EFA. The horizontal line represents no inhibition in cell growth, i.e. same number of viable cells as control cultures. Viable cells were determined by trypan blue dye exclusion assay. For concentrations of 0.01 to 0.5 μg/ml the proliferative response was significantly different from control, $P < 0.02$; for concentrations of 3 to 100 μg/ml the inhibitory response was significantly different from control, $P < 0.01$.

of this conditioned medium containing about 2, 5, or 10 U of IL-2/ml did not prevent lymphocytolysis of CEM-C7 cells treated with 10 nM Dex for 4 days.

Response to exogenous interleukin-2

To test more specifically for the involvement of IL-2 in glucocorticoid-induced cell lysis, CEM-C7 cells treated with 10 nM Dex were provided concentrations of rat splenocyte IL-2 from 5 to 325 U/ml (activity verified as described in Experimental) (Table 1A). These concentrations of IL-2 augmented the Dex inhibitory effect. In the original experiment by Smith annd Gillis [3] demonstrating the ability of IL-2 to overcome Dex-induced inhibition of mitogen-activated lymphocyte proliferation, the amount of IL-2 in the conditioned medium was not indicated, but it may be surmised to be about 10 U IL-2/ml medium. However, perhaps a lower concentration of commercial IL-2 or Jurkat conditioned medium + continuous conditioned medium from human lymphocytes could prevent glucocorticoid-induced cell death. Table 1B shows that neither 1 U IL-2/ml alone nor Jurkat conditioned medium (~ 50 U IL-2/ml) + 1 U IL-2/ml reversed the cell lysis or the inhibition of tritiated thymidine uptake due to 10 nM Dex treatment on CEM-C7 cells cocultured with human lymphocytes. To enable a potentially protective mechanism of IL-2 to function better, addition of 1 U/ml IL-2 24 h prior to Dex was also tested [45]. Figure 3 demonstrates that tritiated thymidine uptake in CEM-C7 was severely inhibited by 10 nM Dex and that time of glucocorticoid delivery relative to IL-2 was not a factor.

Having tested the hypothesis that suppression of glucocorticoids are mediated by suppression of essential lymphokine production, we conclude that glucocorticoid resistance in the CEM-C7 leukemic clone is not conferred by exogenous IL-2 or any other secreted growth factors in the conditioned media produced from glucocorticoid-sensitive or glucocorticoid-resistant, or IL-2 secreting cells. The direct, receptor-mediated cell lysis of lymphoid and leukemic cells by glucocorticoids must occur via some other mechanism.

Biphasic response to PGE analogs

Prostaglandins are rapidly inactivated *in vivo* and *in vitro*, but certain synthetic analogs, such as enisoprost (E) and enisoprost free acid (EFA) have greater potency and longer duration of action. The response of CEM-C7 cell growth to E or EFA was biphasic, depending upon prostaglandin concentration. After 4 days' treatment, there was a statistically significant, 20% stimulation of cell growth (increased viable cells)

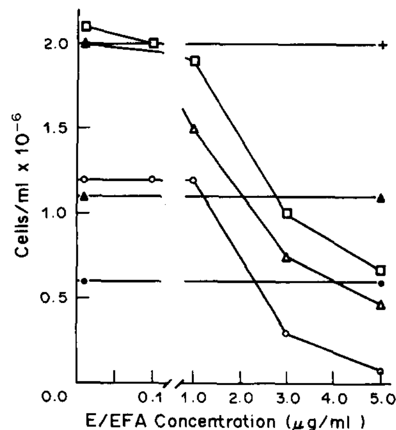

Fig. 5. The interactive effects of E-EFA with Dex on the growth of CEM-C7 cells. All cultures were initially 1×10^5 cells/ml; control cultures treated with vehicle only; after 4 days viable cells were determined by trypan blue dye exclusions assay from duplicate values of triplicate samples. Three horizontal lines are used for ease of visual comparison to represent viable CEM-C7 cells in control cultures and reduced number of viable cells with two *single* concentrations of Dex: control cultures(+), 10 nM Dex (▲), 30 nM Dex (●). The effect of E/EFA alone in increasing concentrations is shown by the curve connecting open boxes. Since both prostaglandin analogs produced equivalent results, the data were combined. Concentrations of E/EFA used were 0.01, 0.1, 1, 3, and 5 μg/ml (□). Combinations of Dex and the same concentrations of E/EFA can be compared with Dex alone by following the curves connecting corresponding open symbols to the closed symbol Dex only data: 10 nM Dex + E/EFA (△) and 30 nM Dex + E/EFA (○).

at concentrations of 0.001 to 0.5 μg/ml (2.6 nM–1.3 μM). At higher concentrations of either compound (3 to 10 μg/ml, 7.9–26 μM) there was increasing inhibition of growth, leading to cell death at concentrations of 10 μg/ml (Fig. 4). This biphasic response of prostaglandins in tissue culture supports the hypothesis that arachidonic acid metabolites are concentration dependent intracellular regulatory molecules [46–48]. The proliferative response of CEM-C7 cells to E is in the physiological range of natural PG's (1–10 nM) [49] and may indicate that this synthetic compound is acting analogously to a natural prostaglandin involved in lymphoid cell growth. However, it has been shown that normal peripheral blood mononuclear cells (PBMC) do not exhibit a proliferative response to low concentrations of E, and thymidine uptake is inhibited by 22% at 1 μg/ml E [50]. The same group reported that ⩾0.1 μg/ml of E suppressed IL-2 production and responsiveness in PBMCs. The observation of a proliferative response to low concentrations of E as well as the absence of IL-2 protection from glucocorticoid-induced lysis in CEM-C7 cells may indicate a different prostaglandin mechanism for leukemic vs normal lymphoid cells.

Biphasic effects of prostaglandins were also seen in the response of CEM-C7 cells to E/EFA combined with Dex. The lower concentrations of E (0.01 to 1 μg/ml) antagonized the growth-inhibiting effect of low doses of Dex. At higher concentrations of E (⩾3 μg/ml), both E and Dex exerted inhibitory effects on CEM-C7 cells' growth (Fig. 5). The results obtained from treating CEM-C7 cells with fourteen different concentrations of E or EFA and Dex were assessed statistically using an analysis of variance procedure for a two-factor factorial experiment at the 0.05 significance level. At 10 or 30 nM Dex, the growth inhibitory effects of the higher concentrations of E/EFA (3–5 μg/ml) were found to be additive to those of the steroid.

There are reports identifying synergistic responses between glucocorticoids and prostaglandins in lymphoid cells [25, 51, 52]. To our knowledge this is the first study showing concentration dependent interactions in leukemias between the antiproliferative response to glucocorticoids and prostaglandin levels. As to the antagonistic effects at low Dex and E/EFA concentrations, it is not clear if the growth enhancing prostaglandin effect is overcoming the glucocorticoid response or if the Dex is cancelling the proliferative effect of the prostaglandin. The additive cell growth inhibitory effect of high concentrations of E and Dex may indicate separate concentration dependent pathways.

Thus the glucocorticoid-induced lymphocytolysis in this human leukemic T-cell line may be modified biphasically by PGE prostaglandins, depending on their concentration. However, IL-2 or components in these conditioned media had no effect in ameliorating the lethal response to glucocorticoid.

Acknowledgements—This work was done partly in conjunction with the Walls Medical Research Foundation and was supported in part by grants to E. B. Thompson from NIH/NCI (CA 41407) and the G. D. Searle Company. We wish to thank David G. Chilton and John S. Smith for graph preparation, and Julie Farrington, Sarada Kunapuli and Chad Graber for technical assistance.

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