Comparison of Glucocorticoid-Induced Effects in Prolactin-Dependent and Autonomous Rat Nb2 Lymphoma Cells (43622)

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Abstract. **Cultured Nb2 node rat lymphoma cells require lactogenic hormone for their proliferation. We reported previously that dexamethasone (Dex) inhibits prolactin (PRL)-induced mitogenesis and, in the absence of mitogen, induces apoptosis of Nb2 cells. Both antiproliferative and cytolytic effects of Dex on Nb2 cells appear to involve glucocorticoid (Type II) receptor mediation. In this study, we compared Dex effects in PRL-dependent Nb2 cells (Nb2) with SFJCD1 (SF), a clone of Nb2 cells that proliferates independently of exogenous PRL. Proliferative assays involved a 72-hr incubation in a chemically defined, serum-free medium where ovine PRL (1 ng/ml) was added to Nb2 cells but not to SF cells. Both cell lines were responsive to the antiproliferative effects of Dex in a dose (6.25–200 n*M*)-dependent fashion of comparable sensitivity and magnitude. Co-incubation with the glucocorticoid receptor antagonist, RU 486, prevented the antiproliferative effect of Dex in both cell lines. In the same medium devoid of PRL, Dex was cytolytic to Nb2 cells and fragmented DNA in a fashion reflective of apoptosis, but was ineffective in SF cells. A dual chamber incubation system revealed no evidence that SF cells produced cytokines that were mitogenic or anticytolytic to Nb2 cells. Both Nb2 and SF cells fragmented DNA in a fashion indicative of apoptosis in the presence of the Ca^{2+} ionophore, A23187 (1 μM). These studies reveal a basic difference in glucocorticoid responsiveness between the PRL-dependent Nb2 cell line and its PRL-independent subclone, SF. While both cell lines exhibit functional glucocorticoid receptors and the necessary intranuclear machinery for apoptosis, the pathway mediating the latter is inhibited or dysfunctional in SF cells.** [P.S.E.B.M. 1993, Vol 203]

The Nb2 node lymphoma cell line, phenotypically classified as an early thymocyte, was originated in an estrogen-treated male rat and has been perpetuated in cell suspension culture (1–3). Cultured Nb2 cells require prolactin (PRL) or other lactogenic hormones for mitogenesis, a characteristic of the parent tumor, and will give rise to PRL-dependent tumors when injected subcutaneously into Nb rats (3). As a result of their specific requirement for and high sensitivity to PRL, Nb2 cells have been used as an *in vitro* bioassay system for the measurement of lactogens in body fluids (4). In addition, the cell line has served as a model for the study of mechanisms of action of PRL (5–10).

Since lymphoid tissue is known to be a target for glucocorticoids (11–14), we initiated studies of the PRL-dexamethasone (Dex) interaction in Nb2 cells. We reported that while Dex inhibited PRL-induced Nb2 cell proliferation, via the glucocorticoid (Type II) receptor, the cells were resistant to cytolytic effects of Dex in the presence of mitogen. In the absence of PRL, however, Dex produced cytolysis, also mediated by the glucocorticoid receptor. This Dex-induced cytolysis involved apoptosis, or programmed cell death, as reflected by the characteristic internucleosomal fragmentation of genomic DNA. On the other hand, PRL protected the cell against Dex-induced apoptosis, a property specific for hormones with lactogenic activity. As we discussed previously, this mutual inhibitory interaction between lactogenic and glucocorticoid hormones on Nb2 lymphoma cells reflects a complex relationship that does

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not appear to be explained on the basis of one hormone's modulation of the other's receptors (15).

In addition to the PRL-dependent Nb2 cell, subclones of this line exist that do not require PRL, or apparently any other known exogenous mitogen, to promote cell proliferation (16, 17). The purpose of this study was to examine the effects of dexamethasone in a PRL-independent subline of Nb2 cells (SFJCD1), relative to PRL-dependent cells.

Materials and Methods

Hormones. Ovine prolactin (oPRL S-15) was provided as a gift by the National Hormone and Pituitary Program. Dexamethasone (1,4-pregnadiene-9-fluor-16α-methyl-11β,17α,21-triol-3,20-dione) was obtained from Sigma Chemical Co., St. Louis, MO. RU 486 (11-β-(-4-dimethylaminophenyl)-17β-hydroxy-17α-(prop-1-ynyl)estra-4,9-dien-3-one) was provided as a gift by Roussel Uclaf (Paris, France).

Maintenance of Lymphoma Cells. Two sublines of Nb2 cells were examined in this study. The PRL-dependent cells, Nb2 clone 2, were from the same source used by us in a previous study (15) and are designated herein as Nb2 cells. The PRL-independent cell line was subline SFJCD1 (designated herein as SF), a subclone of the parent PRL-dependent line (16, 17), and was provided as a gift of Dr. Peter Gout, Cancer Control Agency of British Columbia.

Both Nb2 and SF cells were resurrected from storage in liquid nitrogen. Both cell lines were maintained in tissue culture flasks in Fischer's medium supplemented with 10% horse serum, 50 units/ml of penicillin, 50 μg/ml of streptomycin, and 0.1 m*M* 2-mercaptoethanol (pH 7.4). Nb2 cell medium was additionally supplemented with 10% fetal calf serum (FCS), the source of lactogens (4, 15), in a water-saturated atmosphere of 5% CO_2 and 95% room air at 37°C (standard atmospheric conditions), while SF cells were maintained in the above medium devoid of FCS supplement under the standard atmospheric conditions, as reported previously (16, 17) and recommended by Dr. Gout. Unless otherwise specified, all tissue culture medium reagents were purchased from Gibco BRL Life Technologies, Grand Island, NY.

Proliferative Assay. The proliferative assay was conducted to examine the antiproliferative effects of dexamethasone and was a modification of the standard mitogenic assay described previously (4, 15). Both sublines were washed twice by centrifugation (300*g* for 5 min at 10°C) and resuspension of pelleted cells in Fischer's medium (unsupplemented), followed by a third centrifugation and resuspension in preincubation medium. Cells were then preincubated in tissue culture flasks for 24 hr at a concentration of 0.5×10^6 cells/ml. Nb2 cells were preincubated in the above-described Fischer's incubation medium (supplemented with horse serum, antibiotics, and 2-mercaptoethanol), where the FCS was reduced to 1% to retard growth (4, 15), while SF cells were preincubated in this medium devoid of FCS.

The assay incubations were conducted in 24-well plates at a final plating cell concentration of 1×10^5 cells/ml in a chemically defined, serum-free medium in a volume of 1 ml. This medium was prepared by supplementing Fischer's medium with the following constituents: 2-mercaptoethanol, penicillin, streptomycin, Hepes, bovine serum albumin, linoleic acid, pyruvate, transferrin, selenium, vitamins, amino acids, spermidine, and $CaCl_2$ in previously reported concentrations (18). The steroid hormones (Dex and/or RU 486) were dissolved in dimethyl sulfoxide such that final vehicle concentration was 0.25% (a concentration that was shown previously to affect neither Nb2 cell mitogenesis nor viability) (15). Nb2 cells were incubated under standard atmospheric conditions in the presence of oPRL (initially dissolved in 0.01 *N* NaOH and diluted in incubation medium) at a concentration of 1 ng/ml, a maximal mitogenic dose for the hormone, while SF cells were incubated in the above medium devoid of PRL. Cell cultures were incubated in duplicate for 72 hr under standard atmospheric conditions and cell concentrations were measured with a Coulter counter as described previously (15).

Cytolytic Assay. The cytolytic assay employed in the current study was a modification of the assay reported previously, where cell viability was measured after a 24-hr incubation in medium deficient in lactogenic hormone (15). Nb2 and SF cells were obtained from their respective maintenance conditions and washed twice as described above. Cells were then resuspended in the above-referenced serum-free, chemically defined incubation medium (devoid of PRL) and were incubated for 24 hr in 6-well plates at a concentration of 0.5×10^6 cells/ml in the presence of the cytolytic agents dexamethasone or A23187 (Sigma) or the vehicle dimethyl sulfoxide (at 0.25%) under standard atmospheric conditions. When cell viability and cell number were the principal end points, the volume of cells incubated was 2 ml. However, when DNA fragmentation was also measured, the incubation volume was 7 ml. At the end of incubation, cell viability and cell number were measured, respectively, by trypan blue exclusion and a Coulter counter (15).

For measurement of DNA fragmentation, a protocol was adapted from previously reported methods (19–21). A 6-ml aliquot of the above incubation mixture ($3–4 \times 10^6$ cells) was pelleted by centrifugation (300*g* for 5 min at 10°C) and the supernatant media was removed. The pelleted cells were resuspended in 1.5 ml of lysing buffer (5 m*M* Tris, 5 m*M* EDTA, and 0.5% Triton X-100 [pH 7.5]) and incubated on ice for

20 min with periodic vortexing. An aliquot of the lysate was then centrifuged at 13,000 rpm for 15 min and DNA measured in the supernatant and pellet by the diphenylamine reaction (22). DNA fragmentation was estimated on the basis of amount of DNA that did not pellet (i.e., released from the genome).

Evaluation of DNA Fragmentation by Agarose Gel Electrophoresis. Nb2 and SF cells were obtained from their respective maintenance media and washed twice, as described above. The cells were then resuspended in the above-referenced chemically defined incubation medium at 0.5×10^6 cells/ml in 75-cm^2 tissue culture flasks containing either Dex (100 n*M*), A23187 (1 μM), or vehicle (0.25% DMSO), and 30 ml were incubated under standard environmental conditions for 24 hr. Cells were pelleted by centrifugation (1000 rpm for 5 min at 10°C) and processed for DNA analysis by agarose gel electrophoresis, as described previously (12, 15).

Dual Chamber Experiments. In the course of these investigations, the question arose as to whether SF cells secreted a cytokine that stimulated cell proliferation and/or offered protection against the cytolytic effects of Dex. The rationale for these studies was that if such substances influenced SF cells, they would also influence the parent line, Nb2 cells. Therefore, a dual incubation system was implemented in which the material released from SF cells was tested for its effect on cell number and viability of Nb2 cells. The dual incubation system enabled the physical separation of the two cell populations that shared a common incubation medium. This system was composed of 6-well multidishes in which were placed 25-mm tissue culture inserts (Nunc, catalog no. 1-62138). The inserts are polystyrene cylinders (11 mm in height) containing a porous inorganic membrane across the bottom and elevated on 1-mm legs. Placement of the insert inside the multidish well produces two chambers, an outer (the original well) and an inner (the insert), where media mixture occurred through the porous membrane. The treatments of interest (Nb2 or SF cells, hormones, vehicles) were placed in the outer chamber in a volume of 2.5 ml, while the response of Nb2 cells (proliferation or viability) in a volume of 2.0 ml was monitored in the inner well.

Proliferative assays conducted in the dual incubation system used the same preincubation and incubation conditions as single-well proliferative and cytolytic assays. Nb2 cells were plated in the inner chamber at 1×10^5 cells/ml. The conditions of the outer chamber, which vary, are described in the Results. As in the single-well assays, Coulter counts were obtained after a 72-hr incubation period.

Cytolytic assays in dual chamber experiments involved incubation of cells in chemically defined, serum-free medium. Nb2 cells were plated in the inner chamber at 0.5×10^6 cells/ml. The conditions of the outer chamber are described in the Results. As in the single-well cytolytic assays, cell viability was estimated by trypan blue exclusion after a 24-hr incubation period.

Statistics. Data from individual experiments conducted on different days were used to generate means and SE. Treatments were compared statistically by analysis of variance and Duncan's multiple range analysis, as reported previously (15).

Results

Antiproliferative Effects of Dex on Nb2 and SF Cells. Figure 1 shows that in the absence of Dex, SF cells that were incubated in lactogen-free, serum-free, chemically defined medium achieved a cell concentration after 72 hr, which is about 25% greater than Nb2 cells. The latter were incubated in the same medium supplemented with a maximal stimulatory dose of oPRL (1 ng/ml). Both cell lines were responsive to the antiproliferative effects of Dex in a dose-dependent fashion, and the sensitivity and magnitude of the antiproliferative effects in both cell lines appear comparable.

Figure 2 shows that co-incubation of 100 n*M* Dex with 500 n*M* RU 486, the glucocorticoid receptor antagonist, prevented the antiproliferative effect of Dex in both cell lines.

Cytolytic Effects of Dex in Nb2 and SF Cells. Figure 3 shows the effects of 100 n*M* Dex on cell viability of Nb2 and SF cells incubated in the lactogen-

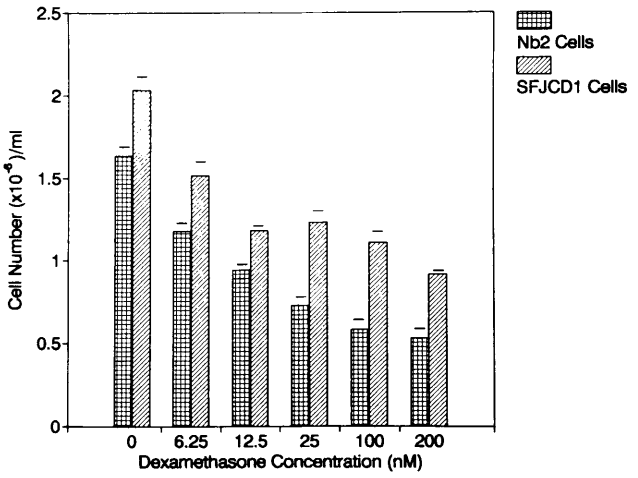

Figure 1. Effect of Dex on mitogenesis of Nb2 and SF cells after 72 hr of incubation in a serum-free, chemically defined medium. Nb2 cells were incubated in the presence of oPRL (1 ng/ml), while SF cells were incubated in the absence of exogenous hormone. Cells were plated at 1×10^5 cells/ml. Each bar and corresponding line represent mean and SE, respectively, of six separate incubations. In the absence of exogenous oPRL, the concentration of Nb2 cells obtained was $0.25 \pm 0.01 \times 10^6$ cells/ml. For either cell line, cell numbers at all concentrations of Dex were significantly less than the respective control (0 Dex) incubations ($P < 0.01$) and cell numbers at the higher Dex concentrations (100 and 200 n*M*) were significantly lower than that at 25 n*M* Dex ($P < 0.05$).

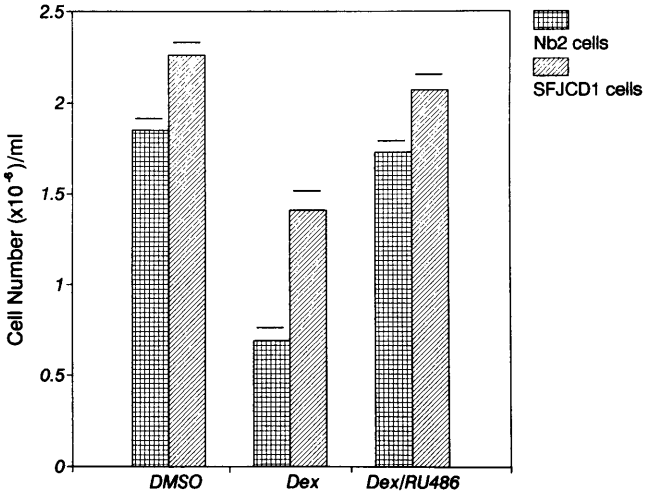

Figure 2. Effect of co-incubating the glucocorticoid receptor antagonist, RU 486 (500 n*M*), with Dex (100 n*M*) on mitogenesis of Nb2 and SF cells. Conditions were the same as those described for Figure 1. Each bar and corresponding line represent mean and SE, respectively, of three separate incubations. For either cell line, cell numbers with Dex treatment were significantly lower ($P < 0.01$) than dimethyl sulfoxide (DMSO) and Dex/RU 486, while DMSO and Dex/RU 486 did not differ significantly from one another.

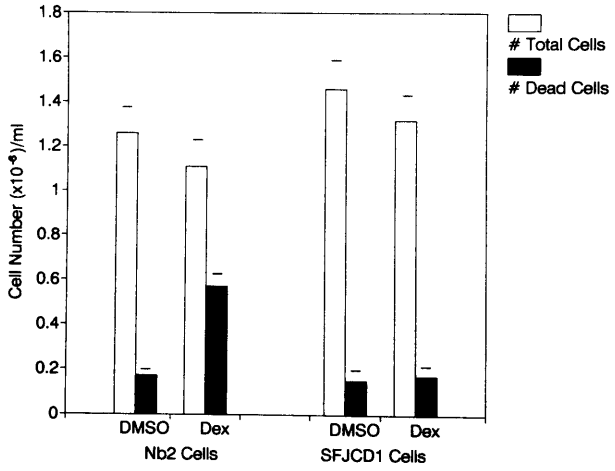

Figure 3. Effect of Dex (100 n*M*) on cell viability of Nb2 and SF cells after 24 hr of incubation in a serum-free, chemically defined medium (devoid of PRL). Each bar and corresponding line represents mean and SE, respectively, of eight separate incubations. Dead cell number for Dex-treated Nb2 cells was significantly greater ($P < 0.01$) than that for dimethyl sulfoxide (DMSO)-treated Nb2 cells.

free, chemically defined medium for 24 hr. While 100 n*M* Dex produced a significant increase in the percentage of dead cells in the Nb2 line, which is consistent with our previous report (15), SF cells were resistant to the cytolytic effect of Dex. In neither cell line did Dex produce a significant change in the total number of cells as compared with controls under cytolytic assay incubation conditions.

Figure 4 shows that the effects of 100 n*M* Dex on DNA fragmentation (i.e., DNA released from the genome) in Nb2 and SF cells are consistent and quantitatively comparable with those observed for cell viability. While Dex produced an increase in DNA fragmentation in Nb2 cells, no significant effect of Dex on DNA fragmentation was observed for SF cells. No significant effects for Dex on total DNA content were observed in both cell lines.

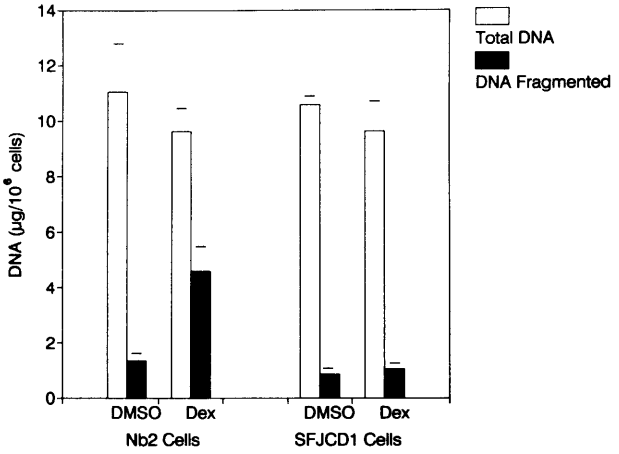

Figure 4. Effect of Dex (100 n*M*) on DNA fragmentation of Nb2 and SF cells after 24 hr of incubation in a serum-free, chemically defined medium (devoid of PRL). DNA fragmented is the amount of DNA remaining in the supernatant after lysing of cells and centrifugation of lysate. Total DNA is the sum of the DNA measured in the supernatant and pellet. Each bar and corresponding line represents mean and SE, respectively, of three separate incubations. DNA fragmented in Dex-treated cells is significantly greater ($P < 0.01$) than that for dimethyl sulfoxide (DMSO)-treated Nb2 cells.

Dual Chamber Assay for the Detection of a Mitogenic and/or Anticytolytic Cytokine Produced by SF Cells. The behavior of SF cells to Dex was similar to how Nb2 cells responded to the steroid when PRL was present: inhibition of mitogenesis without increased cytolysis (15). This suggested that SF cells were producing a PRL-like cytokine that stimulated mitogenesis and offered protection against the cytolytic effects of Dex. The existence of such a putative cytokine(s) was tested in a dual incubation system in which the influence of medium containing SF cells was tested on cell number and viability of Nb2 cells.

Table I shows the results of the experiment that tests the effect of varying concentrations of SF cells (outer chamber) on Nb2 cell number (inner chamber). PRL addition (with Nb2 cells) in the outer chamber is capable of stimulating proliferation of Nb2 cells in the inner chamber (Treatments 1–4), indicating that the hormone was capable of diffusing across the porous barrier and eliciting a mitogenic effect. However, the addition of SF cells to the outer chamber at plating concentrations of $1–5 \times 10^5$ cells/ml (Treatments 5–7) was incapable of stimulating proliferation of Nb2 cells in the inner chamber.

Table II shows the results of experiments to test whether SF cells produce a protective cytokine. Dex added in the presence of SF cells (Treatment 4 versus 3) was just as cytolytic to Nb2 cells in the inner chamber

Table I. Effect of SFJCD1 Cells on Mitogenesis of Nb2 Cells Using Dual Chamber Design[a]

Treatment	Treatment[b] (outer chamber)	Response (inner chamber)
1	Nb2 cells (1×10^5/ml)	0.22 ± 0.02
2	Nb2 cells (1×10^5/ml) + oPRL (0.5 ng/ml)	1.20 ± 0.25[c]
3	Nb2 cells (1×10^5/ml) + oPRL (1.0 ng/ml)	1.48 ± 0.17[c]
4	Nb2 cells (1×10^5/ml) + oPRL (2.0 ng/ml)	1.53 ± 0.30[c]
5	SF cells (1×10^5/ml)	0.24 ± 0.01
6	SF cells (2×10^5/ml)	0.29 ± 0.04
7	SF cells (5×10^5/ml)	0.26 ± 0.02

[a] Data are means ± SE of three individual incubations representing total number $\times 10^{-6}$/ml of Nb2 cells after 72 hr of incubation.
[b] Number in parentheses represents plating concentration of cells at the start of incubation.
[c] $P < 0.01$ versus all other treatments.

Table II. Effect of SFJCD1 Cells on Dexamethasone-Induced Cytolysis of Nb2 Cells Using Dual Chamber Design[a]

Treatment	Treatment (outer chamber)	Response (inner chamber)
1	Nb2 cells + DMSO	13.3 ± 4.1
2	Nb2 cells + Dex (100 n*M*)	55.5 ± 2.5[b]
3	SF cells + DMSO	16.5 ± 2.9
4	SF cells + Dex (100 n*M*)	61.5 ± 2.2[b]

[a] Data are means ± SE of four individual incubations representing percentage of dead Nb2 cells after 24 hr of incubation.
[b] $P < 0.01$ versus respective dimethyl sulfoxide (DMSO) control.

as Dex added in the presence of Nb2 cells (Treatment 2 versus 1).

In these dual chamber experiments, cell number and viability were also measured in the outer chambers. Nb2 cells and SF cells behaved in a fashion identical to that observed in single-well assays (data not shown).

Cytolytic/Apoptotic Effects of A23187 and Nb2 and SF Cells. The resistance of SF cells to Dex could also reflect the absence in this cell line of the basic mechanism for Dex-induced cytolysis, namely the endonuclease within the nucleus of the cell that fragments the DNA in a distinct pattern, indicative of internucleosomal cleavage (a marker for apoptosis). Since the calcium ionophore, A23187, has been shown previously to produce such cytolysis in lymphoid tissue, presumably by activating a Ca^{2+}-Mg^{2+}-dependent endonuclease (20, 21), the effects of this substance in SF and Nb2 cells were compared with Dex.

Figure 5 shows that Nb2 cells exhibit DNA fragmentation in response to either Dex (100 n*M*) or A23187 (1 μM), whereas SF cells are resistant to the cytolytic effects of Dex but elicit significant DNA fragmentation in response to the ionophore. Agarose gel

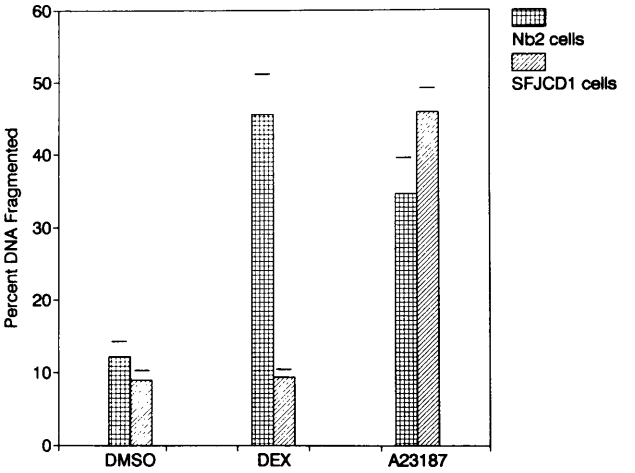

Figure 5. Effect of Dex (100 n*M*) or A23187 (1 μM) on percentage of DNA fragmented of Nb2 and SF cells after 24 hr of incubation in a serum-free, chemically defined medium (devoid of PRL). Each bar and corresponding line represents mean and SE, respectively, of four separate incubations. For Nb2, percentage of DNA fragmented for Dex-treated and A23187-treated cells were significantly greater than that of dimethyl sulfoxide (DMSO)-treated cells ($P < 0.01$). For SF, percentage of DNA fragmented of A23187-treated cells was significantly greater than that of Dex-treated and DMSO-treated cells ($P < 0.01$).

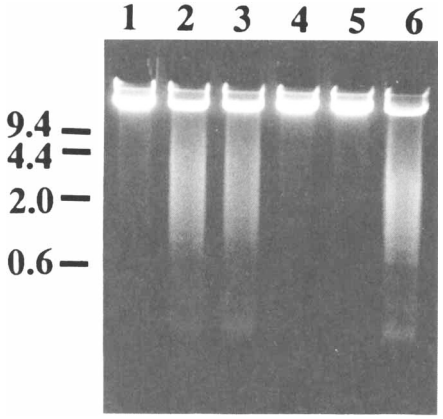

Figure 6. Ethidium bromide-stained UV-irradiated agarose gel of DNA extracted from Nb2 cells and SF cells after incubation with either dimethyl sulfoxide (DMSO), Dex (100 n*M*), or A23187 (1 μM) for 24 hr. Lanes 1–3 are from Nb2 cells and Lanes 4–6 are from SF cells. Lanes 1 and 4: DMSO; Lanes 2 and 5: Dex; Lanes 3 and 6: A23187. Molecular weight markers expressed in kilobase pairs are indicated to the left of the gel. "Ladder" pattern seen in Lanes 2, 3, and 6 reflect internucleosomal DNA cleavage, a marker for apoptosis.

electrophoresis of genomic DNA from Dex- or A23187-treated cells is shown in Figure 6. Both Dex and A23187 produced the characteristic "ladder" pattern of DNA fragmentation in Nb2 cells, while in SF cells this pattern was only seen in genomic DNA obtained from cells treated with A23187, consistent with the data in Figure 5.

Discussion

PRL-independent Nb2 cells were derived by Gout from Nb2 parent cells that were subcultured in medium

deficient in lactogen (16). The small proportion of cells that survived lactogen omission gave rise to a subline that no longer requires PRL for proliferation and, as also seen in the current report, that have a higher proliferation rate than PRL-stimulated Nb2 cells, even when grown in serum-free medium without exogenous mitogen (16, 17). Karyotypic analysis of Nb2 cells and their autonomous sublines, including SFJCD1, reveals that the parent cell has lost one chromosome 17, while among the relatively few changes seen in PRL-independent cells is a translocation of the remaining chromosome 17 with chromosome 14 (17). It has been speculated that, since the rat chromosome 17 harbors the genes for PRL and related proteins, monosomy at this site might underlie PRL dependence, while translocation of the remaining chromosome might underlie PRL independence (17).

In spite of differing conditions for Nb2 and SF cell mitogenic assays (e.g., the presence or absence of PRL, respectively), both cell lines exhibited antiproliferative effects in response to Dex that were comparable in sensitivity and magnitude. In addition, the inhibitory effects of RU 486, the glucocorticoid receptor antagonist, indicate that Dex antiproliferative effects for both cell lines are mediated by glucocorticoid (Type II) receptors (15). It is also noteworthy that the mitogenic effect of PRL and the dose-dependent antiproliferative effect of Dex reported herein are close in magnitude to those reported previously by us (15), even though the previously published experiments were conducted with incubation medium supplemented with serum whereas the current studies used a chemically defined, serum-free medium.

Unlike the PRL-dependent Nb2 cells, SF cells are resistant to the cytolytic/apoptotic effects of Dex. The current study also reveals that the two end points, percentage of dead cells and percentage of DNA fragmented, correspond quantitatively to one another. This quantitative relationship between cell viability and DNA fragmentation at 24 hr is also evident in Nb2 cells under a variety of other experimental conditions (unpublished observations). This suggests that DNA fragmentation assays can be used as alternatives to the more tedious trypan blue exclusion cell viability assays.

The mechanism(s) by which SF cells are resistant to the cytolytic effects of Dex is unexplained. As discussed above, SF cells have functional glucocorticoid (Type II) receptors, and yet, glucocorticoid-induced cytolytic action is in some way blocked. It appears unlikely that SF cells are merely less sensitive to glucocorticoid in general, since both cell lines show similar sensitivity to the antiproliferative effects of Dex. Furthermore, the cytolytic dose of Dex used in this study (100 n*M*) is a relatively strong one (15). In the current series of experiments, cell death and DNA fragmentation were observed in Nb2 cells exposed to Dex doses as low as 6.25 n*M* (data not shown).

Since the behavior of SF cells to Dex is reminiscent of the behavior of Nb2 cells to the steroid when PRL is also present in the medium (15), we tested whether SF cells produced a cytokine that had PRL-like activity, using cell number and viability of Nb2 cells as the end points in a dual chamber incubation system. While the system we used could detect the mitogenic action of PRL and the cytolytic action of Dex when either hormone was added in the adjacent chamber, we found no evidence of cytokine-like substances secreted by SF cells. This is consistent with the previous observations of Gout, who could not demonstrate mitogenic effects of spent medium and/or cellular extracts from PRL-independent cells when tested on parent Nb2 cells (16). Alternatively, Nb2 cells could be hyposensitive to the mitogenic/anticytolytic effects of a PRL-like autocrine substance produced by SF; hence, such a cytokine would be difficult to demonstrate under current bioassay conditions.

A proposed mechanism for apoptosis involves the entry of Ca^{2+} ions into the cell and the activation of Ca^{2+}-Mg^{2+}-dependent endonuclease(s) within the nucleus, which is supported by several lines of evidence, including the apoptotic effect of calcium ionophores (19–21). Our data indicate that SF cells have the requisite machinery to undergo apoptosis, since exposure to A23187 stimulated DNA fragmentation as well as produced the laddering effect stereotypical of apoptosis. In fact, SF cells may be more sensitive to the cytolytic effects of the ionophore, since preliminary data suggest that the threshold for A23187 in SF cells (0.5 μM) may be lower than that required to produce DNA fragmentation in Nb2 cells (1 μM) (data not shown). The ability of SF cells to respond to the antiproliferative effects of Dex and simultaneously be resistant to the apoptotic actions of the hormone suggests that the two glucocorticoid actions employ distinct pathways in which the latter is in some way inhibited in SF cells.

Several gene products have been implicated in modulation of apoptosis. Among these are c-*myc* (23), p53 (24), p35 (25), LMP 1 (26), and *bcl*-2 (26–30). The resistance of SF cells to glucocorticoid-induced apoptosis could be explained either by mutation of a gene product that normally is involved in apoptosis activation or by the induction of a gene product that is inherently anticytolytic. Two reports implicate the *bcl*-2 oncogene, which is associated with inhibition of apoptosis, in a situation similar to that observed herein. Alnemri *et al.* (29) observed that while a pre-B cell line expressing low levels of *bcl*-2 exhibited glucocorticoid-induced antiproliferative effects and apoptotic effects, another pre-B line expressing high levels of *bcl*-2 was resistant to the latter while sensitive to the former. Miyashita and Reed (30) reported that mouse lymphoid

lines transfected with recombinant retrovirus that encoded for *bcl*-2 rendered the cells resistant to Dex-induced apoptosis while retaining responsiveness to glucocorticoid antiproliferative effects. However, in the latter report, a situation exists that is inconsistent with the data reported herein: high *bcl*-2 levels also imparted resistance of the cells to Ca^{2+} ionophore-induced apoptosis (30). In the current study, SF cells respond to ionophore-induced apoptosis while retaining resistance to the cytolytic effects of Dex.

Further studies are underway to understand the control of programmed cell death in PRL-dependent and PRL-independent Nb2 cells.

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