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Symposia

Regulation of Apoptosis in S49 Cells

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Apoptosis, or programmed cell death, is a highly regulated physiological process by which individual cells die and are removed from a given population. This process, defined by both morphological and biochemical characteristics, has been extensively studied in the glucocorticoid-induced immature thymocyte model. In the present study we explore the effects of glucocorticoids on variants of the S49.1 thymocyte without (S49-NEO) or with (S49-bcl-2) the bcl-2 proto-oncogene. In S49-NEO cells dexamethasone induced a time- and dose-dependent loss of viability and increase in DNA internucleosomal fragmentation (a biochemical hallmark of apoptosis). Glucocorticoid treatment was also associated with an apoptotic morphology (cell shrinkage, chromatin condensation) and the effects of this steroid could be reversed by the glucocorticoid antagonist RU486. In contrast, S49-bcl-2 cells showed no change in viability, DNA fragmentation or apoptotic morphology. Interestingly, the apoptotic effects of glucocorticoid in S49-NEO cells were mimicked by the translation inhibitor cycloheximide and the zinc chelator 1,10-phenanthroline, suggesting that zinc and translational events are necessary to maintain the nonapoptotic state. Finally, nuclease activity was extracted from glucocorticoid-treated S49-NEO cells but not control cells. Together the results further define the effects of glucocorticoids on these cells and provide insight into the mechanisms controlling apoptosis.

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INTRODUCTION

Apoptosis is a physiological form of cellular demise in which a given cell is deleted by an orderly and highly regulated series of events (for review see [1, 2]). The death of cells by this process is a widespread phenomenon in biology and its deregulation has been implicated in many pathological conditions including cancer, neurological disorders and AIDS. Despite its importance, however, work on apoptosis has lagged far behind that of other disciplines. Apoptosis is characterized by very distinctive and conserved morphological and biochemical events. Morphological markers of this process include shrinkage of the cell and condensation of chromatin around the periphery of the nucleus. Eventually the entire cell buds into small spherical structures called apoptotic bodies. These apoptotic bodies are endocytosed by neighboring cells and/or resident macrophages, effectively deleting the cell without eliciting an unwanted immune response. Prior to these morphological changes, biochemical alterations occur. The preeminent biochemical change in apoptosis is the activation of an endogenous endonuclease which cleaves the genomic DNA in the internucleosomal or linker DNA regions. Cleavage in these regions produces fragments of nucleosomal or oligonucleosomal lengths. These fragments then form a banding or "ladder" pattern when subjected to agarose gel electrophoresis. Activation of this nuclease has been suggested to be the initial, irreversible event leading to eventual cell death [3, 4].

While this process has been identified in a variety of cell types, the best studied model is the glucocorticoid-induced death of immature thymocytes. In this model, treatment of adrenalectomized rats with glucocorticoids causes a rapid loss of thymocytes by apoptosis, resulting in a 50% reduction in thymic mass over 2 days [5]. This rapid loss can be accounted for solely by

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the death of 80% of the immature medullary lymphocytes [5], whereas the more mature cortical thymocytes, epithelia and stromal elements remain unaffected. Our lab is interested in studying this process and the operative mechanisms that control its initiation. Moreover, because of the suggested importance of the nuclease in the induction of apoptosis, we are highly interested in the mechanisms which control the activity of this enzyme. *In vivo* studies aimed at investigating intracellular mechanisms suffer severely by the inability to strictly control the levels of test substances reaching their targets. Therefore, such an *in vivo* model as that described above would be inappropriate for these studies. Accordingly, we took advantage of the S49.1 mouse thymoma cell line which appears to retain the characteristics of thymocytes *in vivo*, including the sensitivity to glucocorticoids [6]. In the present study we characterize apoptosis in variants of these cells that have been transfected with the neomycin-resistance gene alone (S49-NEO) or in combination with the proto-oncogene bcl-2 (S49-bcl-2). This oncogene has previously been shown to inhibit apoptosis in many systems including the glucocorticoid-induced death of thymocytes [7]. Additional experiments are performed to gain insight into the mechanisms which may regulate the nuclease activity and apoptotic process in these cells.

MATERIALS AND METHODS

Reagents

Tris (hydroxymethyl) aminomethane (Tris) and sodium dodecyl sulfate (SDS) were obtained from Fisher Scientific (Norcross, GA) whereas (ethylenedinitrilo)-tetraacetic acid (EDTA) was received from EM Science (Curtin Matheson Scientific, Houston, TX), chloroform from Mallinckrodt Chemicals (Paris, KY) and phenol from United States Biochemical (Cleveland, OH). Proteinase K was purchased from Boehringer Mannheim (Indianapolis, IN) and RU486 was kindly provided by Dr Martini, Roussel-UCLAF (Romainville, France). Joklik's Minimum Essential Medium (JMEM) was obtained from Flow Labs (McLean, VA) while RPMI 1640 medium was received from JRH Bioscience (Lenexa, KS). All other chemicals, including RNase A, dexamethasone, 1,10-phenanthroline and cycloheximide were obtained from Sigma Chemicals (St Louis, MO).

Cell culture

S49-NEO and S49-bcl-2 cells are stable transformants of the parental S49.1 cell line. These cells were developed by electroporation of wild-type cells with a recombinant amphotropic retrovirus carrying the neomycin resistance gene alone (S49-NEO) or in combination with a bcl-2 cDNA sequence (S49-bcl-2) as described previously [8]. Both cell types were maintained (37°C; 93% air/7% CO_2) in logarithmic growth (10^5–10^6 cells/ml) in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 4 mM glutamine, 100 U/ml penicillin, and 75 U/ml streptomycin sulfate. For experiments, cells were cultured at 5×10^5 cells/ml in the above media containing 5% fetal calf serum for the indicated times. HeLa cells were grown in suspension culture (37°C) in JMEM supplemented with 1% fetal calf serum, 1% calf serum, 2 mM glutamine, 75 U/ml penicillin and 50 U/ml streptomycin sulfate.

Viability determination

Viability of cells at the end of experiments was determined by the ability to exclude trypan blue. Cells were mixed 1:1 with trypan blue (final concentration 1%), incubated 5 min at room temperature and counted in a hemacytometer. At least two fields were counted and care was taken to distinguish between shrunken apoptotic cells (which were counted) and cellular debris (which was not).

Preparation of nuclear extracts

S49-NEO cells were counted, pelleted and resuspended in nuclei isolation buffer (10 mM $MgCl_2$, 0.25% Nonidet P-40). Nuclei were then collected by centrifugation and resuspended (0.5 ml/2.5×10^8 cells) in extraction buffer [0.3 M NaCl, 1 mM EDTA, 20 mM Tris (pH 7.4)] for 1 h (4°C) with rotation. Extracts were then cleared of chromatin and other debris by ultracentrifugation (165,000 *g*, 1 h, 4°C). Protein concentrations were measured by the method of Bradford [9] and extracts stored at −20°C until used.

DNA isolation and analysis

For DNA integrity analysis, 5×10^6 cells were pelleted, lysed in 5 mM Tris (pH 8.0), 20 mM EDTA, 0.5% Triton X-100 and treated with 0.4 mg/ml proteinase K for 1 h at 55°C. Lysates were then extracted twice with phenol–chloroform–isoamyl alcohol (25:24:1, by vol.) and once with chloroform–isoamyl alcohol alone. NaCl was added to 100 mM and DNA precipitated by the addition of 1 ml ice-cold 100% ethanol. DNA pellets were dried and resuspended in 10 mM Tris (pH 7.4), 1 mM EDTA containing 0.3 mg/ml DNase-free RNase A. Following overnight incubation at 37°C, DNA concentrations were measured by absorbance at 260 nM and samples (15 μg) subjected to electrophoresis (80 V, 3.25 h) on 1.8% agarose gels. Gels were then stained with ethidium bromide and visualized by UV transillumination.

HeLa nuclei assay

This assay was performed essentially as described previously [10]. Briefly, HeLa cells were counted, resuspended in nuclei isolation buffer and nuclei pelleted. Nuclei were then resuspended in buffer

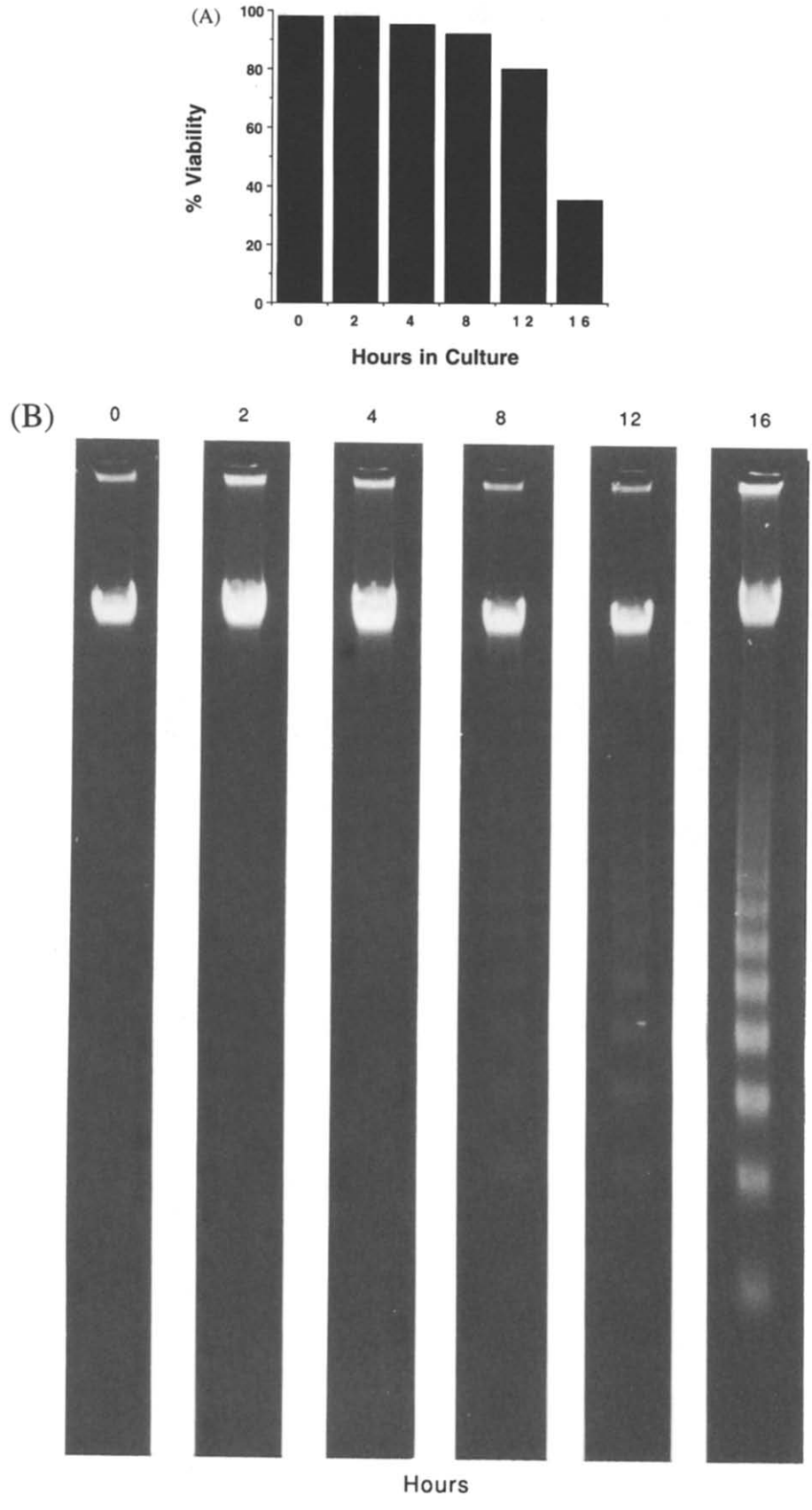

Fig. 1. Time course of DEX (100 nM) effects on S49-NEO cells. Viability measurements are from a single representative experiment repeated at least three times (variability at 16 h approx. 20%) whereas gels are from the identical experiment. (A) Viability measurements as determined by trypan blue exclusion. (B) DNA integrity analysis. DNA was isolated at each time point, as described in Materials and Methods, and subjected to agarose gel electrophoresis and ethidium bromide staining.

containing 50 mM Tris (pH 7.4), 1 mM $CaCl_2$, 1 mM $MgCl_2$ and experimental treatments. Nuclei (10^6/sample) were then incubated for 90 min at 25°C and the reaction terminated by the addition of NaCl, EDTA and SDS to 540 mM, 25 mM, and 0.5%, respectively. DNA was then isolated and subjected to agarose gel electrophoresis as described.

Histology

For histological analysis, cells were pelleted, resuspended in methanol–acetic acid (3:1, v/v; 10 min) and dried onto glass slides. Slides were subsequently stained with hematoxylin and eosin and photographed with standard light microscopic techniques.

RESULTS

As an initial experiment we investigated the kinetics of dexamethasone (DEX) effects on S49-NEO cellular viability and DNA integrity. As shown in Fig. 1(A), S49-NEO cells lost the ability to exclude trypan blue as a function of the length of exposure to 100 mM DEX. While the loss occurs slowly at first (4% during the first 8 h), it increases dramatically following the 8 h point (60% loss during the last 8 h). Indeed, the major loss of viability (70% of total) occurred during the last 4 h of culture, suggesting an initial lag phase during which the steroid associates with its receptor and alters transcriptional events, eventually leading to the rapid decline in viability. This loss of viability occurs through the induction of apoptosis [Figs 1(B), 2(B) and 3]. Figure 1(B) shows a clear time-dependent appearance of apoptotic DNA fragmentation with the oligonucleosomal pattern apparent as early as 8 h, substantially prior to the rapid loss of viability. Such a temporal relationship between DNA fragmentation and loss of viability is characteristic of the process of apoptosis. Because of the large differences between initial and final conditions at 16 h this time point was chosen for further study.

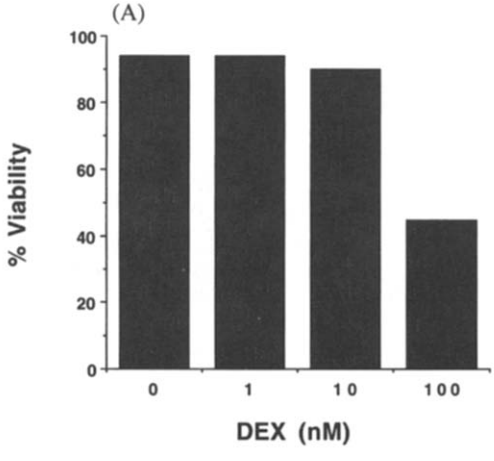

Fig. 2(A)

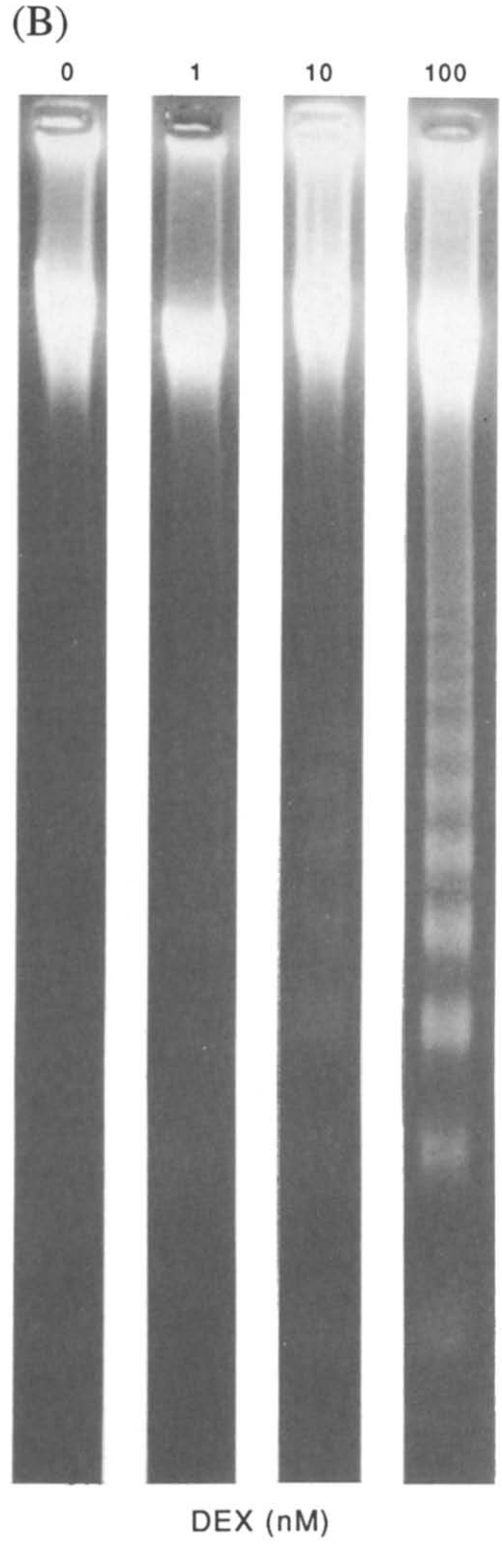

Fig. 2(B)

Fig. 2. Effect of increasing concentrations of DEX on S49-NEO cells following 16 h of culture. Viability measurements are from a single representative experiment repeated at least three times (variability at 100 nM DEX approx. 20%) whereas gels are from the identical experiment. (A) Viability measurements. (B) DNA integrity analysis.

Figure 2(A) shows the viability of S49-NEO cells cultured for 16 h in the presence of increasing concentrations of DEX (0–100 nM). There was little or no loss of viability with 1 nM DEX and only a slight decrease when cells were treated with 10 nM of the steroid. However, 100 nM DEX caused a dramatic loss of viability to approx. 40%. Using this data we have estimated an ED_{50} of 90 nM, which is in agreement with the K_d for DEX interaction with the glucocorticoid receptor [11]. As shown in Fig. 2(B), there is a dose-dependent increase in the amount of oligonucleosomal length DNA fragments extracted from these cells, confirming that the effects on viability are correlated with a biochemical marker of apoptosis.

As described above, apoptosis is defined in morphological as well as biochemical terms. Therefore analysis of cellular morphology is essential for accurate determination of the apoptotic process as a mechanism for the loss of viability. Figure 3 shows the morphology of S49-NEO cells incubated 16 h with 100 nM DEX. Control cells (CON) show large nuclei with dispersed chromatin whereas cells treated with DEX appear shrunken with condensed chromatin in nuclear fragments. The fragmented nuclei are in the early stages of the formation of apoptotic bodies. These features are the morphological hallmarks that define apoptosis.

Regulation of DNA fragmentation and nuclease activity

To investigate the need for functional steroid receptors in the response of S49-NEO cells to glucocorticoids we utilized the glucocorticoid antagonist RU486. As shown in Fig. 4, during a 16 h incubation DEX stimulated the appearance of DNA fragments in S49-NEO cells. However, the DEX-induced increase in DNA fragmentation is suppressed by a 10-fold molar excess of the glucocorticoid antagonist RU486 (Fig. 4), demonstrating that these effects are exerted directly through the glucocorticoid receptor. The slight apoptotic-like changes seen with RU486 + DEX can be accounted for by an incomplete competition of the antagonist with the agonist or, more likely, a partial agonist activity of RU486.

Apoptosis is a genetically controlled event and much work is currently underway to identify the genes which regulate this process. One gene that has clearly been shown to be involved is the bcl-2 proto-oncogene. This gene has been shown to inhibit apoptosis in many systems, including the glucocorticoid-induced death of thymocytes [7]. To investigate if this gene would suppress apoptosis in S49 cells we obtained variants which stably express the bcl-2 gene on the same neomycin resistance containing vector used to generate S49-NEO cells [8]. As shown in Fig. 4, the effects of DEX on DNA fragmentation can be completely suppressed by the overexpression of the bcl-2 gene. Recent data has shown that bcl-2 appears to act in the regulation of oxygen free radical metabolism [12]. The ability of this gene to completely inhibit DEX-induced apoptosis suggests the intriguing possibility that DEX may in some way initiate apoptosis through the regulation of these species.

Previous studies have suggested that the nuclease is present in nonapoptotic cells but is maintained in a latent state by association with a proteinaceous inhibitor [13, 14]. Furthermore, this inhibitor appears to have a shorter half-life than the enzyme. To investigate this possibility, cells were treated with the translation inhibitor cycloheximide (CX; 1 μM; Fig. 4). While treatment with CX suppresses DEX-induced apoptosis [15], the drug alone causes cells to eventually (Fig. 4 shows 24 h of CX treatment) acquire the DNA fragments of apoptosis, suggesting that the enzyme is associated with a labile repressor which degrades with faster kinetics than the nuclease, subsequently activating the nuclease and inducing the apoptotic cascade.

Because of the importance and temporal appearance (prior to morphological changes and loss of viability) of the nuclease activity in apoptosis, a series of experiments were undertaken to investigate the nature of the responsible protein. Many nucleases are inhibited by zinc and this ion has been shown to inhibit apoptosis in a variety of systems [16]. In addition, recent data from our lab [13] has shown that Zn^{2+} can inhibit

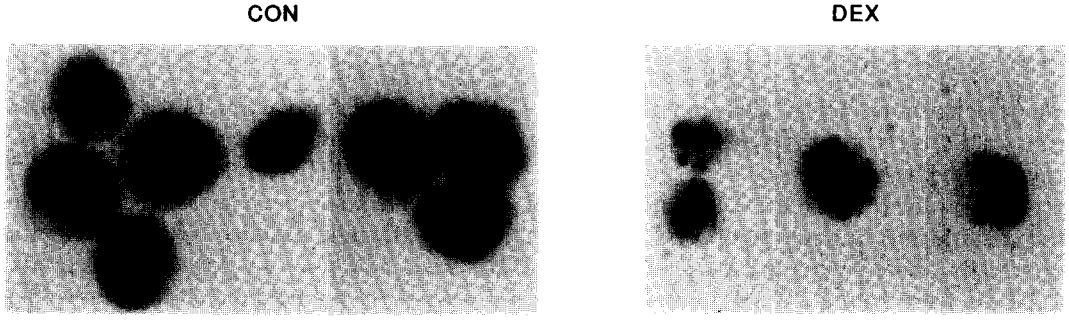

Fig. 3. Morphology of S49-NEO cells following 16 h of incubation with (DEX) or without (CON) 100 nM DEX. Cells were resuspended in methanol–acetic acid (3:1, v/v 10 min), dried onto glass slides, stained with hematoxylin and eosin and photographed at 1000× magnification. Similar results have been seen in multiple experiments.

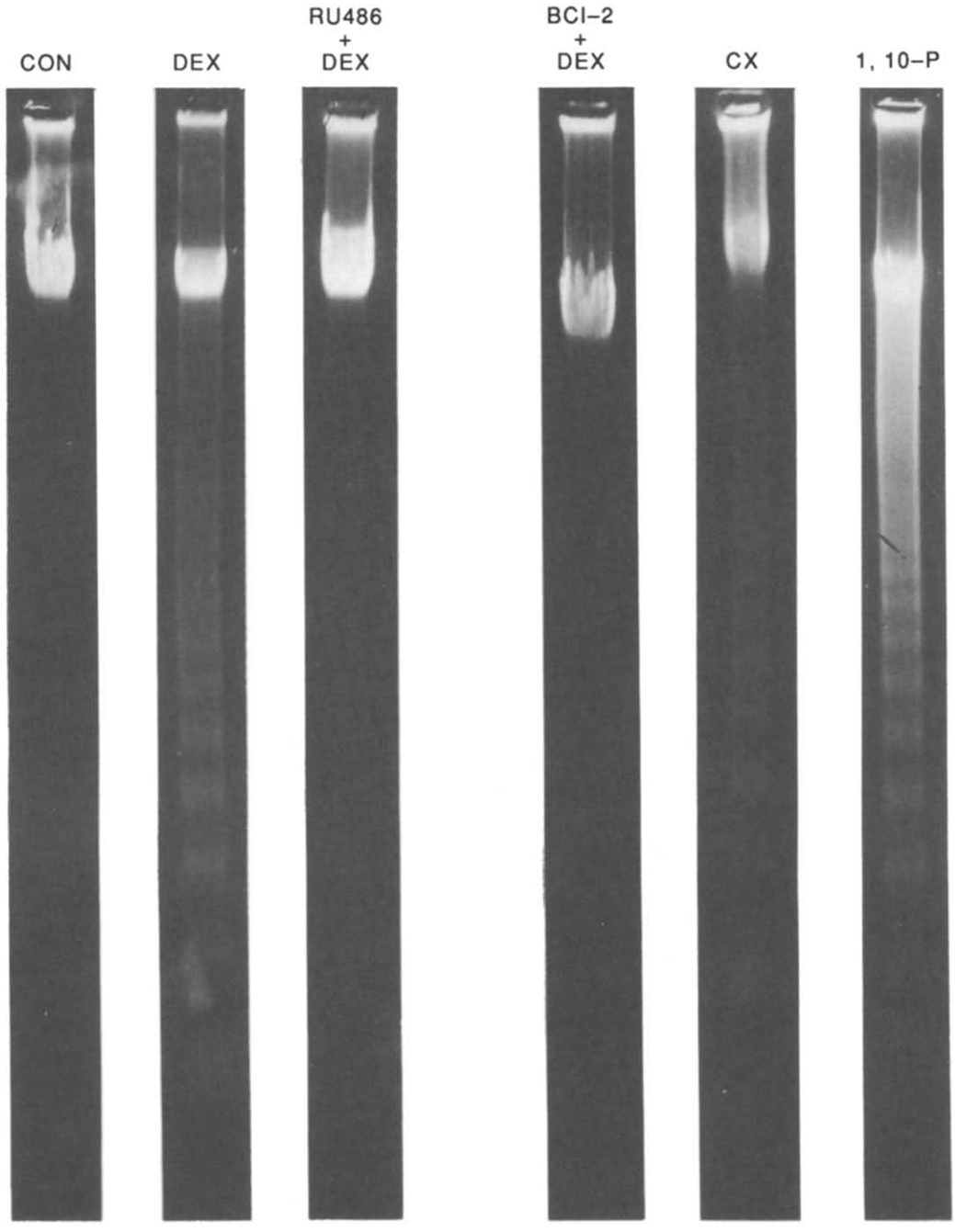

Fig. 4. Effects of treatments on DNA integrity in S49-NEO or S49-bcl-2 cells following 16 or 24 (CX only) h of incubation. Gels are from representative experiments repeated two or more times with similar results. The abbreviations are: CON, control; DEX, 100 nM dexamethasone; RU486, 1 μM RU486; bcl-2, S49-bcl-2 cells: 1,10-P, 0.5 mM 1,10-phenanthroline; CX, 1 μM cycloheximide.

in vitro internucleosomal DNA cleavage activity extracted from apoptotic thymocytes, suggesting a direct role for this ion in the inhibition of nuclease activity. Therefore, we reasoned that removal of Zn^{2+} should activate the enzyme and initiate the apoptotic cascade. Accordingly, cells treated with the intracellular Zn^{2+} chelator 1,10-phenanthroline (0.5 mM) experienced a dramatic increase in DNA fragmentation (Fig. 4), suggesting that Zn^{2+} functions intracellularly in a constitutive fashion to inhibit nuclease activity and, consequently, apoptosis.

Finally, nuclear extracts from apoptotic thymocytes have been shown to possess internucleosomal cleavage activity [10, 13]. Similar extracts from DEX-treated S49.1 cells have been associated with nuclease activity able to degrade naked DNA [17]. However, the ability of such S49 nuclear extracts to degrade intact chromatin with an internucleosomal specificity has never been explored. Therefore, in the present experiment we extracted the nuclease activity from control and DEX-treated S49-NEO cells and examined their nuclease activity in the HeLa nuclei assay developed

previously in our lab. This assay recapitulates the *in vivo* configuration of DNA and thus provides a suitable substrate for analysis of internucleosomal cleavage activity. Cleavage of DNA in the internucleosomal regions generates DNA fragments which form an apoptotic-like oligonucleosomal ladder on an agarose gel. As shown in Fig. 5, extracts from control S49-NEO cells (CON) were unable to degrade HeLa DNA, however, when nuclear extracts were derived from DEX-treated cells (DEX), these extracts evoked the oligonucleosomal banding pattern indicative of apoptotic nuclease activity. Therefore, nuclease activity in these cells is extractable in a manner similar to that used to extract nuclease activity from apoptotic thymocytes [10, 13]. This enzyme should now be amenable to further *in vitro* studies.

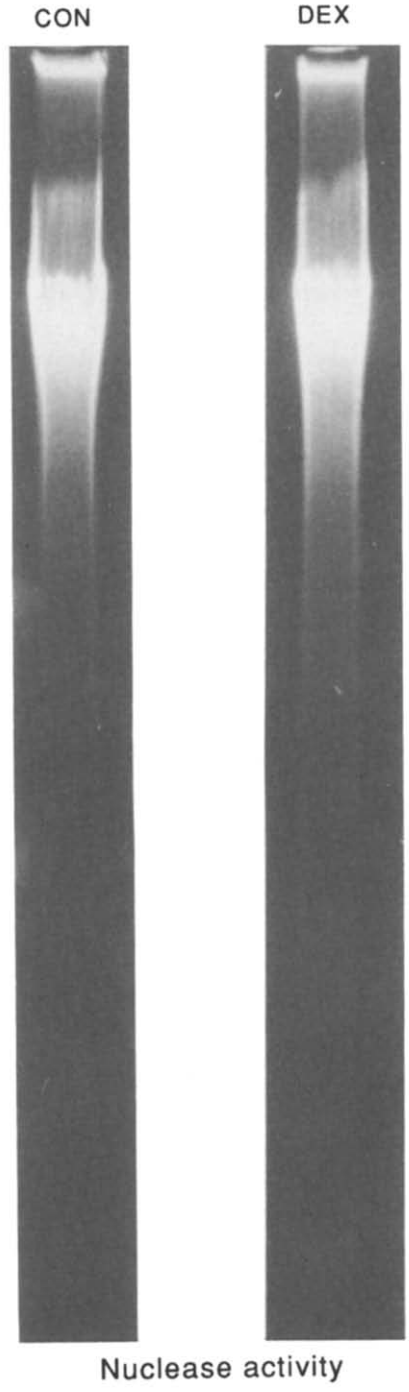

Fig. 5. HeLa assay of nuclear extracts obtained from S49-NEO cells incubated 16 h in the presence (DEX) or absence (CON) of 100 nM DEX. Gels are from representative experiments repeated three times with similar results.

DISCUSSION

Most models of apoptosis display the same conserved morphological and biochemical characteristics, suggesting that a common underlying mechanism governs these changes irrespective of cell type. The majority of knowledge regarding such common mechanisms has been accumulated using the glucocorticoid-induced immature thymocyte model. In the present study we have further characterized the effects of DEX on viability, DNA integrity and cellular morphology in the S49.1 immature thymocyte cell line. Accordingly, we detected a time- and dose-dependent loss of viability, temporally associated with a stimulation of both morphological and biochemical markers of apoptosis, by the synthetic glucocorticoid DEX. Moreover, the effects of this steroid could be inhibited by RU486 and bcl-2. In addition, the apoptotic program was initiated by CX and 1,10-phenanthroline alone. Finally, active nuclease was extracted from DEX-treated but not control cells. Taken together the present results further explore the role of DEX in thymocyte apoptosis and provides insight into potential mechanisms that may regulate nuclease activity in these cells.

The ability of bcl-2 to inhibit DEX-induced apoptosis in the S49 variant we employed was not altogether unexpected, but in light of new evidence [12] on the mode of action of bcl-2 it suggests a novel mechanism for the activity of this steroid. Specifically, glucocorticoids may function in the regulation of oxygen radical metabolism. Although in these initial studies [12] DEX did not increase the measurable amount of oxygen radical generation or peroxide production, it did cause a significant amount of lipid peroxidation. These results would suggest that DEX was not directly effecting the production of oxygen radicals but rather altering their metabolism by shifting their targets (toward lipid peroxidation, etc.). This intriguing mechanism of steroid action is certainly worthy of further study.

One particularly interesting result in this study is that obtained with the Zn^{2+} chelator, 1,10-phenanthroline. Zn^{2+} has been shown to inhibit apoptosis in a number of systems and we have shown that it inhibits the internucleosomal cleavage activity prepared from apoptotic thymocytes [13]. These *in vitro* experiments suggest that Zn^{2+} may bind directly to the enzyme, perhaps displacing a bound Ca^{2+} or Mg^{2+}, and inhibiting its activity. However, the recent demonstration [12] of bcl-2's involvement in oxygen radical metabolism provides an alternative, or complementary, mechanism for Zn^{2+} inhibition, that is through the copper-zinc

superoxide dismutase (Cu/Zn SOD). This enzyme converts superoxide, O_2^-, to H_2O_2 which can then be metabolized to water. Thus, addition of Zn^{2+} would enhance the activity of this enzyme, controlling the availability of oxygen radicals and presumably the activation of DNA fragmentation and apoptosis. Conversely, removal of Zn^{2+} by chelation could disable the Cu/Zn SOD and allow the accumulation of high levels of oxygen radicals which act to induce apoptotic cell death. In this regard it is interesting that Zn^{2+} inhibits apoptosis in a large number of systems with a wide range of inducing agents; a range of activity similar to that of bcl-2. Thus it is possible that both agents may act along a common pathway in apoptosis.

The present results demonstrate the time- and dose-dependent effects of DEX on viability, internucleosomal DNA fragmentation and morphology in S49-NEO cells. These effects could be reversed by the glucocorticoid antagonist RU486 or the proto-oncogene bcl-2 and mimicked by the translation inhibitor CX or the zinc chelator 1,10-phenanthroline. In addition, nuclease activity was extracted from apoptotic cells and analyzed by an *in vitro* nuclease assay. Taken together the present results further characterize the S49.1 thymoma cell line variant S49-NEO as well as provide significant insight into the nuclease activity inherent to apoptosis in these cells. The ability to extract this nuclease and maintain its internucleosomal specificity will make it amenable to further *in vitro* studies.

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REFERENCES

1. Schwartzman R. A. and Cidlowski J. A.: Apoptosis: The biochemistry and molecular biology of programmed cell death. *Endocrine Rev.* **14** (1993) 133–151.
2. Waring P., Kos F. J. and Mullbacher A.: Apoptosis or programmed cell death. *Med. Sci. Res.* **11** (1991) 219–236.
3. Compton M. M. and Cidlowski J. A.: Rapid *in vivo* effects of glucocorticoids on the integrity of rat lymphocyte genomic deoxyribonucleic acid. *Endocrinology* **118** (1986) 38–45.
4. Wyllie A. H.: Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* **284** (1980) 555–556.
5. Compton M. M., Caron L. A. and Cidlowski J. A.: Glucocorticoid action on the immune system. *J. Steroid Biochem.* **27** (1987) 201–208.
6. Harris A. W.: Differentiated functions expressed by cultured mouse lymphoma cells: I. Specificity and kinetics of cell responses to corticosteroids. *Expl Cell Res.* **60** (1970) 341–353.
7. Siegel R. M., Katsumata M., Miyashita T., Louie D. C., Greene M. I. and Reed J. C.: Inhibition of thymocyte apoptisis and negative antigen selection in bcl-2 transgenic mice. *Proc. Natn. Acad. Sci. U.S.A.* **89** (1992) 7003–7007.
8. Miyashita T. and Reed J. C.: bcl-2 gene transfer increases relative resistance of S49.1 and WEHI17.2 lymphoid cells to cell death and DNA fragmentation induced by glucocorticoids and multiple chemotherapeutic drugs. *Cancer Res.* **52** (1992) 1–5.
9. Bradford M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye-binding. *Analyt. Biochem.* **72** (1976) 248–254.
10. Schwartzman R. A. and Cidlowski J. A.: Internucleosomal deoxyribonucleic acid cleavage activity in apoptotic thymocytes: detection and endocrine regulation. *Endocrinology* **128** (1991) 1190–1197.
11. Burnstein K. L. and Cidlowski J. A.: Regulation of gene expression by glucocorticoids. *A. Rev. Physiol.* **51** (1989) 683–699.
12. Hockenbery D. M., Oltvai Z. N., Yin X-M., Milliman C. L. and Korsmeyer S. J.: Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* **75** (1993) 241–251.
13. Schwartzman R. A. and Cidlowski J. A.: Mechanism of tissue-specific induction of internucleosomal deoxyribonucleic acid cleavage activity and apoptosis by glucocorticoids. *Endocrinology* **133** (1993) 591–599.
14. Gaido M. L. and Cidlowski J. A.: Identification, purification, and characterization of a calcium-dependent endonuclease (NUC 18) from apoptotic rat thymocytes. *J. Biol. Chem.* **266** (1991) 18,580–18,585.
15. Caron-Leslie L.-A. and Cidlowski J. A.: Evaluation of the role of protein synthesis inhibition in apoptosis in glucocorticoid sensitive and glucocorticoid resistant S49 cells. *Endocr. J.* **2** (1994) 47–53.
16. Duvall E. and Wyllie A. H.: Death and the cell. *Immun. Today* **7** (1986) 115–119.
17. Caron-Leslie L.-A. and Cidlowski J. A.: Similar actions of glucocorticoids and calcium on the regulation of apoptosis in S49 cells. *Molec. Endocr.* **5** (1991) 1169–1179.