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Position 16 of the Steroid Nucleus Modulates Glucocorticoid-induced Apoptosis at the Transcriptional Level in Murine T-Lymphocytes

Mallory Perrin-Wolff, Zohair Mishal,† Jacques Bertoglio* and Marc Pallardy*‡*

*Immunotoxicologie et Cancérogènese, CJF INSERM 93-01 Faculté de Pharmacie Paris-Sud, rue JB Clement, 92296 Châtenay-Malabry, France and †Laboratoire de Cytométrie, CNRS, rue Guy Mocquet, 94805, Villejuif, France

ABSTRACT. Synthetic glucocorticoids (GCs), which possess a different radical substituted in position 16 of the steroid nucleus structure, display various antiproliferative activities on activated lymphoid cells. We analysed this structure-function relationship between dexamethasone (DEX; methyl group in position 16α) and betamethasone (BM; methyl group in position 16β) with regard to two important aspects of GC activity, namely the activation of transcription and induction of apoptosis in IL-2-dependent murine lymphoid cells. DEX induced a higher percentage of apoptotic viable cells compared to BM. This structure-activity relationship was not related to differences in cytosolic glucocorticoid receptor (GR) affinity or kinetics of apoptosis. However, DEX was more efficient than BM in inducing transcriptional activation of an MMTV-CAT plasmid in transiently transfected CTLL-2 cells. In addition, DEX was more potent in inhibiting AP-1 DNA-binding activity compared to BM. These results suggest that the configuration in position 16 may influence the potency of GCs to induce apoptosis in lymphoid cells, mainly by modulating GR-induced transcription. BIOCHEM PHARMACOL 52;9:1469–1476, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. apoptosis; AP-1; betamethasone; dexamethasone; glucocorticoids; immunosuppression

GCs§ are mainly used as clinical tools to suppress both the immune response and processes of inflammation. They exert their effects after binding to a cytoplasmic GR within target cells. The GR is organized in three main domains: the N-terminal domain plays an important role in the transactivation activity of the GR; the C-terminal region contains the hormone-binding domain; and the central basic domain, which contains two zinc fingers, is responsible for the DNA-binding activity of the receptor [1]. GCs, as small hydrophobic molecules, are assumed to diffuse through the cell membrane and to bind to GR thereby allowing the rapid nuclear translocation of the GC-GR complex [2]. Within the nucleus, GR bind as dimers to specific DNA sequences termed GRE upstream of the promoter region of GC-responsive genes [1]. Thus, GC-GR complexes serve as transcription factors that can positively or negatively regulate gene transcription [1, 2].

GCs modulate the immune response by inhibiting gene transcription of cytokines, such as interleukin-1, interleukin-2 (IL-2), interleukin-6, interferon-γ [3, 4] and by inducing apoptosis in T lymphocytes [5]. Although GCs may inhibit cytokine production at several sites, direct interaction of the activated GR with transcription factors, such as AP-1 or NF-κB, resulting in reduced DNA-binding by either protein, could provide a relevant mechanism of action [6–9]. Indeed, the inhibitory effect of dexamethasone (DEX) on IL-2 production is correlated with decreased DNA-binding of both AP-1 and NF-κB to the IL-2 promoter [10].

Apoptosis in various systems is perceived as a suicide process that appears to be genetically controlled. Studies of genes expressed specifically in GC-treated apoptotic immune cells have addressed the issue of the requirement for protein synthesis in this death process [11]. In WEHI-7TG lymphoma cells, 11 genes specifically induced by GCs have been isolated and characterized [12]. RP-2 and RP-8, two other mRNAs associated with GC-induced programmed cell death, have also been described [13]. Furthermore, using stably transfected S49 mouse lymphoma cells, it has been reported that the N-terminal activation domain of the mouse GR is absolutely necessary for steroid induction of lymphocyte apoptosis to take place, suggesting a role for transcription mediated by GR in lymphoid cell death [14, 15].

‡ Corresponding author: Dr Marc Pallardy, CJF INSERM 93-01, Faculté de Pharmacie Paris-Sud, rue JB Clément, 92296 Chatenay-Malabry, France. Tel (33) (1) 46 83 54 92; FAX (33) (1) 46 83 54 96.

§ *Abbreviations:* BM, betamethasone; CAT, chloramphenicol acetyl transferase; DEX, dexamethasone; DPA, diphenylamine; GC, glucocorticoid; GR, glucocorticoid receptor; GRE, glucocorticoid responsive elements; Ho342, Hoechst 33342; Hsp, heat shock protein; IL-2, interleukin-2; MMTV, mouse mammary tumor virus; PI, propidium iodide; RU486, RU38486; TRIAM, triamcinolone.

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The basic chemical structure of GCs consists of a pregnane nucleus with three 6-carbon rings (A, B, C) and a 5-carbon ring (D) [3]. Modifications of this structure, necessary for GC activity, are a ketone oxygen in positions C-3 and C-20, an unsaturated bond between C-4–C-5, and hydroxylation of C-11. In addition, introduction of a 1–2 double bond and fluorination in the 9α position increase the anti-inflammatory effectiveness of GCs, as observed for DEX, BM, and TRIAM. These three molecules (Fig. 1) are also substituted with a different radical in position 16 (CH3α for DEX, CH3β for BM, OH for TRIAM), which eliminates the mineralocorticoid activity but only slightly modifies potency of effects on metabolism and inflammation [3].

Using DEX, BM, and TRIAM, we recently showed that differences in radicals in position 16 may play a role in the induction of apoptosis by GCs in murine IL-2-dependent cells (CTLL-2), but not in murine thymocytes [16]. In CTLL-2 cells, DEX was more efficient in inducing apoptosis than BM which, in turn, was more potent than TRIAM; these observations were correlated to the respective antiproliferative effects of these molecules. In addition, GC-induced apoptosis in these cells needs a functional GR and does not occur in presence of inhibitors of transcription (actinomycin D) or translation (cycloheximide).

To further understand the structure-activity relationship in GC-induced apoptosis on IL-2-dependent cells, we studied the transcriptional activity of GR-DEX and GR-BM complexes in cells transiently transfected with a GC-responsive MMTV-CAT plasmid. Because repressive functions of the GR may also be involved in GC-induced apoptosis in lymphoid cells, we studied AP-1 DNA-binding activity based on the ability of activated GR to inhibit AP-1 activity through protein-protein interactions [6–8]. We report that, in IL-2-dependent cells, GC-induced apoptosis required an active transcription, and that DEX was more efficient in inducing transcriptional activity than BM at all GC concentrations used. These observations were not attributable to differences in affinities for the cytosolic GR or kinetics of apoptosis.

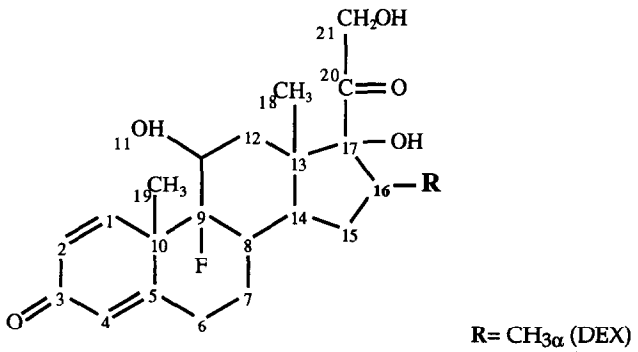

FIG. 1. Structures of dexamethasone and betamethasone.

MATERIALS AND METHODS

Chemicals

DEX, BM, acetyl coenzyme A, Ho342 and PI were purchased from Sigma (Ile d'Abeau, France). RU486 was kindly provided by Roussel Uclaf (Romainville, France). Human recombinant IL-2 was a kind gift from Eurocetus (Amsterdam, The Netherlands).

Cells

Murine IL-2-dependent T-lymphocyte cell lines, CTLL-2 (CD8+, cytotoxic lymphocytes) and HT-2 (CD4+, helper lymphocytes), were cultured in RPMI 1640 medium containing 2 mM L-glutamine, 0.1 mg/mL streptomycin, 100 U/mL penicillin, 10% fetal bovine serum, 5×10^{-5} M 2-βmercaptoethanol, 1% sodium pyruvate, and 1 ng/mL of human recombinant IL-2.

Quantification of Apoptotic Cells by Flow Cytometry

CTLL-2 and HT-2 cells were treated by DEX or BM for 8 hr in the presence of 25 pg/mL IL-2. Cells were washed twice, resuspended at 10^6 cells/mL and incubated for 2 min with 10 μM Ho342 and 32 μM PI [17]. Ho342 was added one min prior to addition of PI. Cells were analyzed using an Epics Elite V (Coulter Electronics, Margency, France). The blue fluorescence of Ho342 was measured through a 460-nm band pass filter and the red fluorescence of PI through a 620-nm long pass filter. Dead cells were highly labeled with PI. Viable normal and apoptotic cells were both lightly labeled with PI but, in contrast to normal cells, apoptotic viable cells were highly labeled with Ho342.

Cytosolic Glucocorticoid-Receptor Binding Assay

Binding assays using [^{3}H]-DEX (Ci/mmol. Amersham, Les Vlis, France) and Scatchard analysis were used to obtain cytosolic GR-binding parameters regarding DEX. To determine GR-binding parameters for BM, competition assays were performed by adding different amounts of cold BM or DEX to a known concentration of radioactive DEX (50 nM) prior to incubation with CTLL-2 cells. CTLL-2 cells were washed twice in PBS 1× and 2×10^6 cells in 0.2 mL of RPMI 1640, 1% BSA, and 0.4% sodium azide, were incubated for 1 h with [^{3}H]-DEX and either cold DEX or BM at 4°C under shaking conditions. CTLL-2 cells were washed 3 times in RPMI 1640 supplemented with 1% BSA and 0.25% sodium azide, lysed in a buffer containing 0.1% Nonidet-40, 3% charcoal, and 0.3% dextran T70, and kept on ice for 10 min. Cell lysates were centrifugated and a cytosolic binding assay was performed by counting the radioactivity in the supernatant using a beta-scintillation counter (Beckman, Gagny, France).

Measurement of DNA Fragmentation

CTLL-2 cells (2×10^6) were lysed in extraction buffer (5 mM Tris-Hcl, 20 mM EDTA, 0.5% Triton X-100) for 30 min at 4°C. Unfragmented DNA was separated from fragmented DNA by centrifugation at 27,000 × *g* for 30 min at 4°C, the supernatant was removed, and the pellet resuspended in extraction buffer. DNA from pellet and supernatant was then precipitated by the addition of 1N perchloric acid. After centrifugation at 27,000 × *g*, the supernatant was discarded, 0.5 N perchloric acid was added, and DNA hydrolyzed by incubation at 70°C. The amount of DNA was quantitated using DPA. Percent of DNA fragmentation refers to the ratio of DNA in the supernatant to the total DNA recovered in supernatant plus pellet.

Electrophoretic Mobility Shift Assay (EMSA)

PREPARATION OF NUCLEAR EXTRACTS. Nuclear extracts were prepared from 10^7 cells by a modification of the method of Dignam *et al.* [18]. Briefly, cells were washed with PBS 1× and resuspended in 2 cell pellet volumes of buffer A (10 mM Hepes pH 7.8, 15 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA, 0.1% Nonidet-40, 1 mM PMSF, 1 mM DTT, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin, 1 μg/mL benzamidine). Cells were incubated 10 min on ice, mixed, and centrifugated. Supernatants were removed and nuclear pellets resuspended in 2 volumes of buffer C (20 mM Hepes pH 7.8, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 25% (v/v) glycerol, 1 mM PMSF, 1 mM DTT, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin, 1 μg/mL benzamidine), and KCl was added in drops to a final concentration of 0.39 M. Nuclei were extracted for 5 min at 4°C and then centrifugated for 20 min at 15,000 × *g* at 4°C. Protein concentrations in the nuclear extracts were evaluated by using the microBCA assay (Pierce, Rockford, IL). For AP-1 DNA-binding assays, end-labelled oligonucleotides were incubated at room temperature for 20 min with 10 μg of nuclear protein in the presence of 2 μg sonicated salmon sperm DNA in 20 μL of binding buffer (12% glycerol, 12 mM Hepes pH 7.8, 60 mM KCl, 1 mM EDTA, 1 mM DTT). Protein-DNA complexes were separated from free probe on a 5% polyacrylamide gel in 0.25× TBE running buffer at 150 V constant. Gels were dried and exposed to X-ray film.

DNA PROBES. Custom-synthesized oligonucleotides were purchased from Bioprobe Systems (Montreuil, France). Complementary sequences were annealed at 80°C for 10 min, 65°C for 10 min, and then end-labelled using [^{32}P]-ATP with T4 polynucleotide kinase (Boehringer, France), and used for EMSA after ethanol precipitation. The 5′ AGCTTGATGAGTCAGCCGGATC 3′ probe was used for AP-1 binding. Specificity was determined by using a 50-fold molar excess of cold AP-1 or mutated AP-1 5′ AGCTTGTGGAGTCAGCCGGATC 3′.

Plasmids, Transfection, and Chloramphenicol Acetyl Transferase (CAT) Assays

MMTV-CAT was used as the reporter CAT plasmid in these experiments as previously described [19]. Cell transfections were carried out by electroporation using a BioRad gene pulser apparatus (960 μF, 250 V). All transfection mixtures contained 10^7 cells in a serum-free medium and 10 μg of plasmid DNA. After electroporation, cells were resuspended in complete culture medium and were treated for 16 hr with various inducers. For determination of CAT activity, cells were collected by centrifugation and lysed by freeze-thawing in hypotonic buffer (250 mM Tris-HCl pH 8). The protein concentration in the cell lysates was determined by using the microBCA assay. Protein extracts were incubated with [^{14}C]-chloramphenicol (specific activity 60 mCi/mmol, Amersham) in the presence of 2 mM acetyl coenzyme A for 1 hr at 37°C. Acetylated chloramphenicol was extracted in ethyl acetate and separated from unmodified chloramphenicol by thin layer chromatography as described in Gorman *et al.* [20]. Following autoradiography, conversion of chloramphenicol was quantitated by cutting and counting spots using a beta-scintillation counter.

Statistical Analysis

Dunett's multicomparison modification of the Student's *t*-test was used to assess the statistical significance of experimental data for continuous variables. Experimental data were considered significantly different from control at $P < 0.05$.

RESULTS

Quantification of GC-Induced Apoptosis on HT-2 and CTLL-2 Cells

To compare the effects of DEX or BM in inducing apoptosis on IL-2-dependent cells, we used a quantitative flow cytometric method based on a double staining with Ho342 and PI. The Ho342/PI method for the quantification of apoptotic cells relies mainly on a change in the permeability of the plasma membrane and allows discrimination between viable normal cells, viable apoptotic cells, and dead dells [17, 21]. Because IL-2 withdrawal induces spontaneous apoptosis in IL-2-dependent CTLL-2 and HT-2 cells, our initial studies were performed in the presence of 25 pg/mL IL-2, a concentration that maintains cells within the cell cycle but does not protect them from GC-induced apoptosis. After 8 hr of incubation, we showed that, in CTLL-2 cells, DEX was the most potent molecule (25% of apoptotic viable cells compared to 12% with BM) to induce apoptotic cell death (Fig. 2). In HT-2 cells, DEX was also significantly more active than BM, with apoptotic cells representing 16% vs 9%, respectively (Fig. 2). These results are consistent with the antiproliferative effects observed with DEX and BM on CTLL-2 and HT-2 cell lines (data not shown).

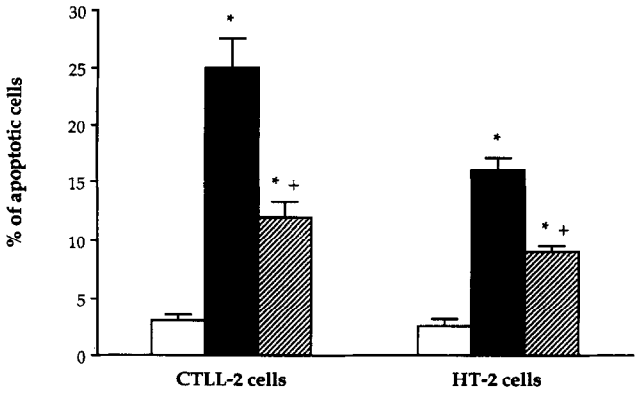

FIG. 2. Quantification of apoptotic CTLL-2 and HT-2 cells by flow cytometry following *in vitro* exposure to DEX or BM. Percentages of apoptotic viable CTLL-2 and HT-2 cells were evaluated following 8-h exposure to GC and human recombinant IL-2 (25 pg/mL). CTLL-2 cells were stained with 10 μM Ho342 added 1 min prior to addition of 32 μM PI. After 2 min staining, 10,000 cells were analyzed using an Epics Elite cytofluorometer. Results are expressed as % of apoptotic viable cells with open bars representing 0 M, bold bars representing 10^{-6} M DEX, and right-hatched bars representing 10^{-6} M BM. Mean of 3 independent experiments. *significantly different from control at $P < 0.05$; + significantly different from DEX-treated cells at $P < 0.05$.

Affinities of DEX and BM for the GC Cytosolic Receptor

Because the results obtained on CTLL-2 and HT-2 cells were comparable, we further analysed the structure-activity relationship observed between DEX and BM on CTLL-2 cells only. We first investigated if this relationship could be related to differences in the affinity of the cytosolic GR for DEX or BM. Using radiolabelled [^{3}H]-DEX and Scatchard analysis, we found 5781 ± 1451 GR sites per cell and a K_d of 8.7 ± 2.2 nM on CTLL-2 cells (Fig. 3A). Results obtained with competition experiments using cold BM or DEX showed a K_d of 6.8 ± 1.9 nM for DEX and a K_d of 8.8 ± 1.6 nM for BM (Fig. 3B). Thus, no significant difference appeared between DEX and BM concerning affinity for cytosolic GR.

Kinetics of DNA Fragmentation Induced by DEX or BM on CTLL-2 Cells

We then investigated if this structure-activity relationship could be explained by a difference in the kinetics of apoptosis induction, which is dependent, among other parameters, on nuclear GR translocation. Using the DPA method in the presence of 25 pg/mL IL-2, we quantified, in a time-course dependent fashion, the percentage of DNA fragmentation induced by DEX or BM on CTLL-2 cells. As shown in Fig. 4, the index relative to the % of DNA fragmentation increased as early as 6 hr following the exposure of CTLL-2 cells to either DEX or BM. In addition, at all time points measured, indexes relative to the % of DNA fragmentation were higher in DEX-treated cells, and kinetics of DNA

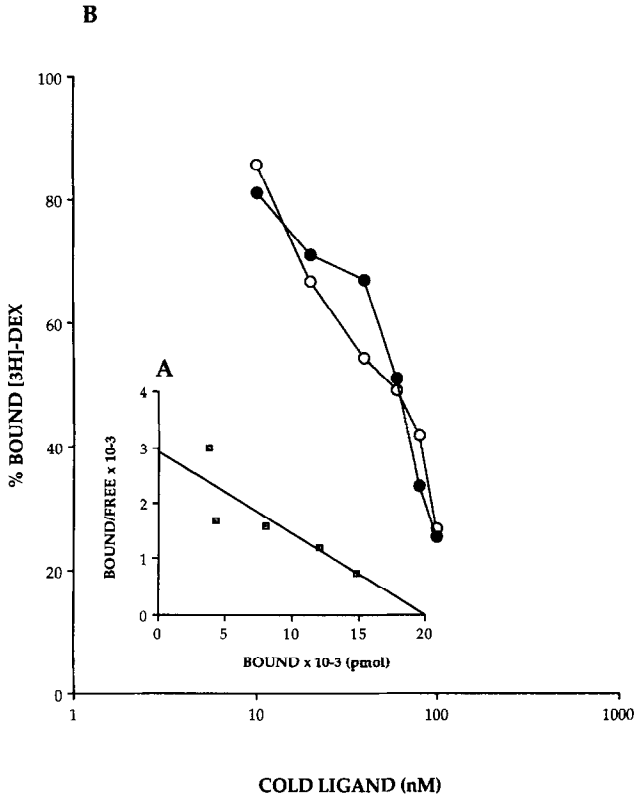

FIG. 3. Affinity of DEX or BM for the cytosolic GR in CTLL-2 cells. (A) Scatchard plot. Cytosolic extracts were prepared from CTLL-2 cells after a 1-hr incubation with different concentrations of [^{3}H]-DEX in the presence or absence of a 1000-fold excess of cold DEX. Specific binding was determined by subtraction of the nonspecific binding from the total binding for each point. This plot is typical of 3 independent determinations. (B) Competition experiments. Cytosolic extracts were prepared from CTLL-2 cells after a 1-hr incubation with 50 nM of [^{3}H]-DEX in the presence or absence of different concentrations of cold DEX (open circle) or BM (bold circle). Specific binding was determined by subtraction of the nonspecific binding from the total binding for each point. This plot is typical of 3 independent determinations.

fragmentation were comparable in cells treated with either DEX or BM.

DEX Induction of a Higher Transcriptional Activity than BM in CTLL-2 Cells Transiently Transfected With an MMTV-CAT Plasmid

Apoptosis induced in CTLL-2 cells following GC treatment may involve transcriptional events mediated by these molecules. Using an MMTV-CAT plasmid transiently transfected in CTLL-2 cells, we evaluated GC-induced transcriptional activity following DEX or BM addition. CTLL-2 cells, immediately after transfection, were treated for 16 hr by different concentrations of GC in presence of 25 pg/mL IL-2. As shown in Fig. 5A, DEX and BM induced a dose-dependent transcriptional activity in these cells, with DEX being the most potent structure. RU486, an an-

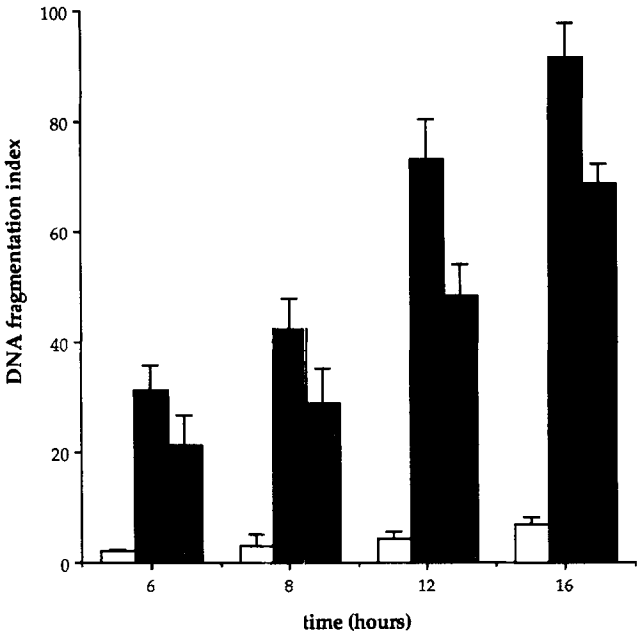

FIG. 4. Kinetics of GC-induced apoptosis on CTLL-2 cells following exposure to DEX and BM. Kinetics of GC-induced DNA fragmentation on CTLL-2 cells were performed using the DPA method in the presence of 25 pg/mL IL-2. Results are expressed using a DNA fragmentation index that represents the ratio between the percentage of DNA fragmentation at the indicated time in the presence of GCs and the percentage of DNA fragmentation at time 0 in the absence of GC. Bars indicate the DNA fragmentation index at the indicated time in the presence of different GC concentrations: 0 M (open bars), 10^{-6} M DEX (bold bars), and 10^{-6} M BM (right-hatched bars). Mean of 2 independent experiments.

tiglucocorticoid, totally abolished transcriptional activity induced by DEX (5.3% vs 91% of chloramphenicol conversion) and was not able, by itself, to positively regulate MMTV-CAT activity (2.3% conversion at 10^{-6} M) (Fig. 5B). Because we previously showed that RU486 protects CTLL-2 cells towards GC-induced apoptosis, these results confirmed that GR transcriptional activity plays a role in CTLL-2 apoptosis after GC treatment [16].

Thus, GCs via GR can induce a dose-dependent transcriptional activity in transient MMTV-CAT-transfected CTLL-2 cells, with DEX being a more potent transcriptional inducer than BM.

AP-1 DNA-Binding Activity After DEX or BM Addition

It has been previously demonstrated that negative regulation of transcription by GCs could be mediated by protein-protein interactions between activated GR and other transcription factors, such as AP-1. We studied the effect of DEX and BM on AP-1 DNA-binding activity in CTLL-2 cells. As shown in Fig. 6, a high basal level of AP-1 DNA-binding activity related to the IL-2 maintenance of CTLL-2 cells was evident using an electrophoretic mobility shift assay. At 10^{-6} M, DEX totally abolished basal AP-1 DNA-binding activity but BM, at the same concentration, only slightly reduced it. Thus, both GR-DEX and GR-BM reduced AP-1 DNA-binding activity, with DEX being the most active molecule.

DISCUSSION

Modifications of the cortisol structure lead to an increase in the ratio of anti-inflammatory to Na^+-retaining potency, so that electrolyte effects are of no serious consequence in number of GC synthetic molecules. Indeed, both methylation (DEX, BM) and hydroxylation (TRIAM) of position 16 on ring D of the steroid nucleus eliminate mineralocorticoid-like effects, but only slightly modify potency of effects on metabolism and inflammation [3]. Using DEX (methyl in 16α), BM (methyl in 16β), and TRIAM (hydroxyl in 16) we previously reported that position 16 strongly modulates GC-induced inhibition of lymphocyte proliferation and apoptosis in CTLL-2 cells [16]. On this IL-2-dependent murine T-cell clone, the activity of these three compounds was found to be ranked as follows: DEX > BM > TRIAM. In the present report, we compared the transcriptional activities of DEX and BM using an MMTV-CAT reporter gene transiently transfected in CTLL-2 cells. We found that the rate of transcription induced by (GR-DEX) and (GR-BM) complexes is correlated with apoptosis induced by these two molecules.

We first quantified apoptotic CTLL-2 or HT-2 cells after DEX or BM treatment using Ho342/PI labelling and flow cytometry analysis. HT-2 cells are IL-2-dependent murine T-helper lymphocytes in which GC have been previously described to induce apoptosis [22, 23]. Our experiments showed that, in both CTLL-2 and HT-2-cells, the percentage of apoptotic viable cells was always significantly higher in DEX-treated than in BM-treated cells, thereby ruling out an idiosyncracy of CTLL-2 cells.

An activated GR is needed for apoptosis to proceed on CTLL-2 cells after GC treatment [16]. Thus, we investigated whether or not some differences could be evidenced in the cytosolic-GR affinity for DEX and BM. The binding affinity of steroids to the cytosolic GR is not always correlated to biological activity. This can be observed with RU486 or 6α, 16α-dimethylprogesterone both of which bind strongly to the GR but are devoid of any biological activity [24, 25]. However, for steroid agonists such as BM or DEX, affinity for the GR is correlated to tyrosine aminotransferase activity or inhibition of uridine synthesis [24, 25]. We did not find any difference in cytosolic-GR affinities between BM and DEX. These results are in agreement with previous studies performed in HTC cells, liver, and thymus cytosol and confirm that affinity for the cytosolic GR is not always discriminating [25].

Kinetics experiments performed in CTLL-2 cells exposed to either DEX or BM showed that, at all time points measured, DEX induced a higher DNA fragmentation than BM. In addition, no shift in kinetics could be shown in these

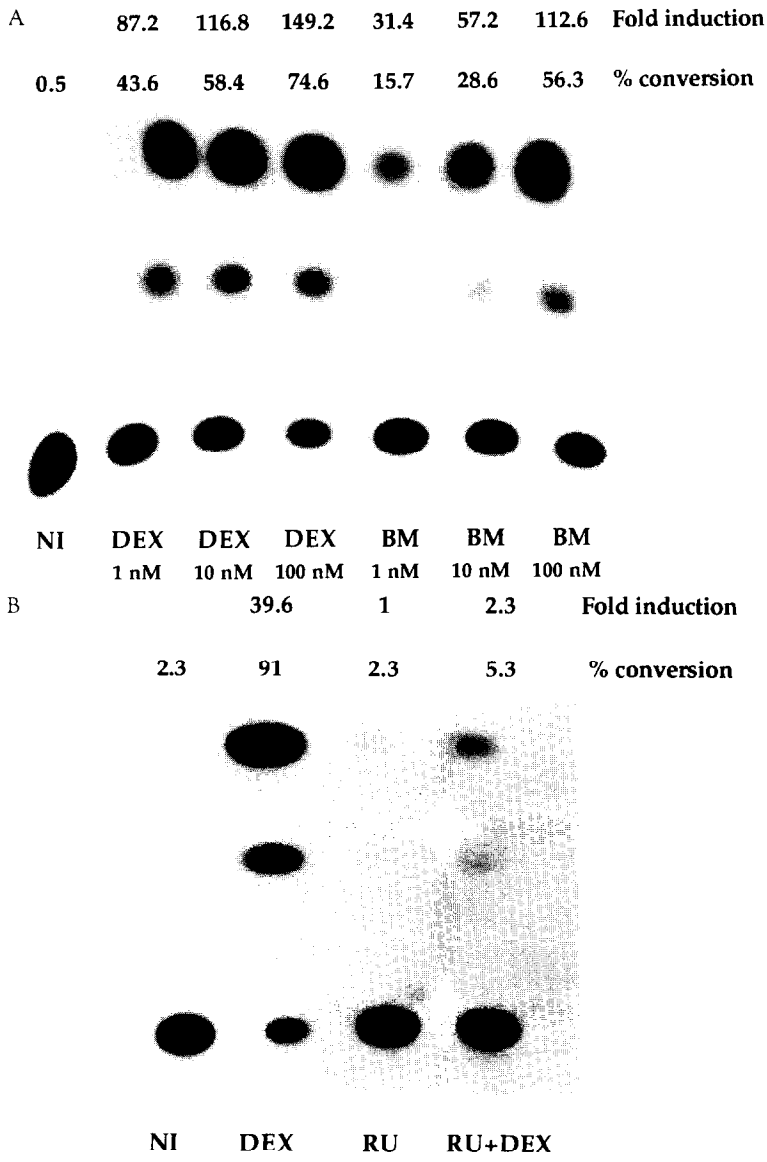

FIG. 5. Regulation of MMTV-CAT transactivation by DEX, BM, or RU486. CTLL-2 cells (10^7) were transfected by electroporation with 10 μg of an MMTV-CAT plasmid. After transfection, the cells were either treated without hormone (NI) or with dexamethasone (DEX), betamethasone (BM), RU486 (RU), or dexamethasone and RU486 (RU + DEX). The % conversion represents the ratio between acetylated chloramphenicol and nonacetylated chloramphenicol. Fold induction is obtained by dividing the % conversion in stimulated cells with the % conversion in untreated cells (NI). (A) Immediately after transfection, cells were incubated for 16 hr in presence of 0 to 10^{-7} M DEX or BM with 25 pg/mL IL-2. Cell extracts were then prepared and assayed for CAT activity. Protein aliquots (10 μg) were used. One representative experiment of 3 is shown. (B) Immediately after transfection, cells were incubated for 16 hr in the presence or absence of DEX (10^{-6} M) and/or RU486 (10^{-7} M) with 25 pg/mL IL-2. One representative experiment of 3 is shown.

experiments, and DNA fragmentation was detectable in both DEX- and BM-treated cells at the 6-hr time point, which corresponds to the point when alteration in DNA structure was first detectable. These results suggest that a critical level of GR has been reached concomitantly in cells treated with either DEX or BM.

GC-induced apoptosis in CTLL-2 cells and in murine thymocytes is an active process requiring receptor translocation and synthesis of RNA and proteins, suggesting that cell death requires transcriptional and translational events [5, 16, 26]. Indeed, RU486, which is known to inhibit GR transcriptional and biological activities [25, 27], protects CTLL-2 cells from DEX-induced DNA fragmentation without inducing cell death by itself [16]. Taken together, these results suggest that, in the CTLL-2 cell model, the transactivation function of the GR is indispensable for the induction of apoptosis. Indeed, in CTLL-2 cells transiently transfected with the MMTV-CAT plasmid, DEX and BM can induce transcriptional activity in a dose-dependent manner, with DEX being the most potent inducer of transcription and RU486 abolishing transcriptional activity mediated by DEX. Transcription from the long-terminal repeat of MMTV has been extensively used to study the regulation of gene expression by GR and contain high affinity binding sites for GR [28]. Thus, these results demonstrate that transcriptional activity is correlated with the effects observed with DEX, BM, and RU486 on apoptosis, suggesting that the structure-activity relationship reported above could be linked to differential potencies in activation of transcription between DEX and BM. In addition, the protective effect of RU486 is likely to be mediated through inhibition of transcription mediated by the GR.

Several parameters could play a role in the differences observed between DEX and BM for their ability to modulate GR transcriptional activity: affinity of the activated GR for GRE sequences, synergism with other transcription factors, and interactions with specific transcription intermediary factors (TIFs). Functional cooperation between

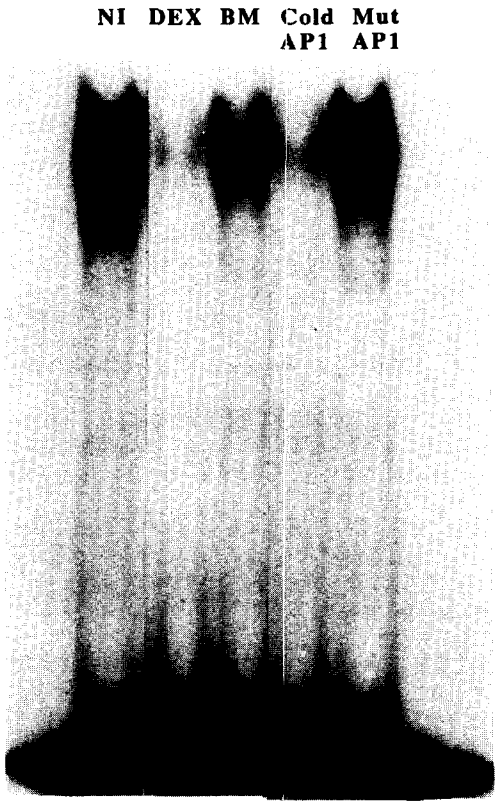

FIG. 6. Effect of DEX and BM on AP-1 DNA-binding activity. Gel retardation assays were performed with a [^{32}P]-labeled oligonucleotide containing the collagenase AP-1 binding site, and nuclear extracts prepared from CTLL-2 cells following 7-hr treatment with 25 pg/mL IL-2 and in the absence (NI) or presence of 10^{-6} M dexamethasone (DEX) or 10^{-6} M betamethasone (BM). Competition reactions were performed with nuclear extracts from untreated cells in the presence of a 50-fold excess of cold or mutated AP-1.

receptor and other transcription factors, such as NF-1 was seen in the MMTV promoter [29]. However, the functional synergism of a GRE with another transcription factor binding site, in contrast to that of a dimerized GRE, cannot be explained by an increase in DNA binding affinity of either factor [30]. Thus, we can hypothesize that the differences observed between DEX and BM on MMTV-CAT transcription may be the consequence of a higher affinity of DEX-GR complexes compared to BM-GR complexes for GRE sequences, or a modulation of protein-protein interactions between GC-GR complexes and the general transcription machinery via TIF protein.

Several studies have shown that apoptosis can still occur in lymphoid cells transfected with a GR lacking the N-terminal domain and devoid of any transcriptional activity [14, 31, 32]. In these studies, repressive functions of the GR were not affected, suggesting that protein-protein interactions between activated GR and other transcription factors involved in cell survival may trigger apoptosis. Indeed, protein-protein interactions resulting in the mutual inhibition of GR or AP-1 function on simple responsive elements have been reported [6–8]. Our results showed that in DEX-treated CTLL-2 cells, AP-1 DNA binding was strongly inhibited compared to cells exposed to BM. IL-2 is known to induce AP-1 activity in CTLL-2 cells and AP-1 is required for cell survival of CTLL-2 cells [33]. In keeping with these findings, whereas GR-induced transcription is a key event for apoptosis to occur in these cells, interference with transcription factors necessary for cell survival may also account for GC-induced apoptosis.

In conclusion, GCs have been used for decades as clinical tools to suppress immune and inflammatory responses, and the majority of structure-activity studies have concluded that GC binding is correlated with the biological activity of the GR agonist. In this study, we have demonstrated that DEX and BM modulate apoptosis on IL-2-dependent lymphocytes differently, although having similar affinities for the GR. Furthermore, our results demonstrate that transcriptional activities induced by DEX or BM play a major role in the potency of these molecules to induce apoptosis, and repression of factors involved in cell survival may play an additional role.

These findings, concerning the configuration in position 16 of the steroid nucleus structure on GC-induced cell death, may be of importance in clinical situations such as treatment of malignant hematological disorders. Indeed, while repressive functions of the GR seems to induce apoptosis in the CEM human leukemic cell model [34], GR-induced transcription is necessary to induce death of thymoma cells [15]. Thus, depending on the maturation stage of lymphocytes, apoptosis induced by GC may require a different mechanism of action.

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