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Glucocorticoid Action on Hybrid Clones Derived from Cultured Myeloma and Lymphoma Cell Lines

(steroid-induced killing/dexamethasone/cytoplasmic receptors)

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ABSTRACT **Cells of a cloned myeloma line from a Balb/c mouse contain specific cytoplasmic glucocorticoid receptors and are killed by dexamethasone. Cells of a lymphoma line (from mouse strain C57BL) also contain specific glucocorticoid receptors but are resistant to the steroid. Cells of two hybrid clones with widely differing chromosome numbers, derived by fusion between the resistant lymphoma and the sensitive myeloma, contain specific glucocorticoid receptors with similar binding properties as the parental receptors and are killed by dexamethasone. Since the lethal effect of the steroid is expressed in the hybrid cells, failure of the parent lymphoma line to be affected by dexamethasone is probably not due to an inhibitor of the lethal reaction.**

The adrenal glucocorticoid hormones have different effects on various differentiated cells. For example, they promote gluconeogenesis and glycogen deposition in liver (1, 2) and induce enzymes in both liver (1, 2) and cultured hepatoma cells (3). They also inhibit the growth of fibroblasts (4, 5) and cause death of some lymphoid cells (6–10). Cells sensitive to glucocorticoids contain specific steroid-binding proteins in their cytoplasm (11–16); binding of the hormones to the receptor proteins is thought to be the first step in all physiological effects of the steroids (11–16). This is illustrated by the fact that steroid-resistant variants derived from sensitive fibroblasts (13) and lymphoma cells (17–19) contain markedly decreased amounts of the specific steroid receptors.

The detailed biochemical steps, beyond the formation of the steroid–receptor complex, that lead to the steroid-induced death of lymphoid cells are not known. One approach to the study of this mechanism is by somatic cell hybridization (20). In this report, we describe the effects of dexamethasone, a synthetic glucocorticoid, on two hybrid clones derived by fusion between cloned Balb/c myeloma cells and C57BL lymphoma cells. Although both parent cells contain comparable amounts of cytoplasmic glucocorticoid receptors, only myeloma cells are susceptible to the lethal effect of the hormone. Furthermore, both hybrid clones, differing greatly in their chromosome numbers, are killed by dexamethasone.

METHODS

Culture Conditions. Cells were grown in Dulbecco's modified Eagle's medium (Grand Island Biological Co.), supplemented with 2 mM glutamine and 1 mM sodium pyruvate under an atmosphere containing 10% CO_2. To each 100 ml of medium, 1 ml of a 100-times concentrated mixture of nonessential amino acids (Grand Island Biological Co.), 100 units of penicillin, 50 μg of streptomycin, and 20 ml of fetal-calf serum (heated for 1 hr at 56°) were added.

Cell Hybridization and Cloning of Hybrid Cells were done as described in ref. 21.

Dexamethasone Binding to Cytoplasmic Receptors. Cells were harvested by centrifugation for 15 min at 2,000 rpm in an MSE (LR-6) centrifuge and washed once with ice-cold 0.1 M sodium chloride–25 mM potassium phosphate buffer (pH 7). Two volumes of 20 mM potassium phosphate buffer (pH 7) were added to the cell pellet, and the cells were disrupted at 0° by 15 strokes in a tissue grinder (Arthur Thomas Co.) with a motor-driven Teflon pestle at 6000 rpm. The homogenate was centrifuged first for 10 min at 10,000 $\times$ g then for 1 hr at 130,000 $\times$ g. The supernatant fluid, called "cytosol," was used for binding assays within 1 hr after centrifugation. The binding of [^{3}H]dexamethasone to cytosol preparations of various cell lines was measured by a modification of a previously described method (11, 12). This method takes advantage of the fact that activated charcoal adsorbs free steroid but not steroid bound to macromolecules. The binding was performed at 0° by incubation of aliquots of cytosol in 20 mM potassium phosphate (pH 7) in a total volume of 300 μl with [^{3}H]dexamethasone, either alone or in the presence of 10 μM unlabeled dexamethasone. As with the glucocorticoid receptors from hepatoma tissue culture cells (11), binding equilibrium was reached by 1.5-2 hr. The incubations were routinely stopped after 3 hr by addition of 50 μl of a suspension of charcoal (100 mg/ml) and agitation for 10 sec in a Vortex mixer. Charcoal was pelleted by centrifugation at 6000 $\times$ g for 15 min. Aliquots of the supernatant fluids were assayed for radioactivity with a Beckman LS-233 scintillation counter at 42% efficiency, and protein was determined by the procedure of Lowry *et al.* (22). All assays were in triplicate. The amount of specifically bound dexamethasone was determined as the difference between charcoal-resistant radioactivity in samples containing [^{3}H]dexamethasone alone and in parallel samples with competing unlabeled steroid (12). Control experiments excluded the possibility that dexamethasone binds to the serum components in the culture medium.

[^{3}H]Dexamethasone (5.8 Ci/mmol) was obtained from Schwarz–Mann and checked for radiopurity by thin-layer chromatography with two solvent systems (23).

RESULTS

Characteristics of the parental cell lines and the hybrid clones

The myeloma parent cell line (CL-4) was an 8-azaguanine-resistant clone derived from the Balb/c mouse myeloma tumor, RPC-5 (24). It synthesized IgG as well as free κ chains and had a modal chromosome number of 60 (see Table 1). The lymphoma parent (EL-4) was a bromodeoxyuridine-resistant cell line (24) derived from a lymphoma induced in a C57BL mouse by 9,10-dimethyl-1,2-benzanthracene (25). It did not synthesize immunoglobulin and had a modal chromosome number of 39 (Table 1). Hybrid clone N5 synthesized only κ chains (21) and had a modal chromosome number of 86, while hybrid clone N6 synthesized IgG and free κ chains (21) and had a modal chromosome number of 100 (Table 1).

Effect of dexamethasone on the parental cell lines and the hybrid clones

The myeloma parent cells (CL-4) were killed by dexamethasone at concentrations of 10 nM or higher (Fig. 1). Although significant killing was observed as early as 24 hr after addition of the steroid, the data shown represent the effect at 48 hr. The observation that CL-4 cells were sensitive to dexamethasone was rather unexpected, since certain myelomas are reported to be insensitive to glucocorticoids (9). However, another myeloma cell line, MOPC-315, when exposed to dexamethasone was also killed (unpublished observation of U. Gehring, D. Givol, and B. Mohit). The lymphoma parent cell line (EL-4) was resistant to 10 μM dexamethasone (Fig. 1). However, both hybrid clones were sensitive to the steroid (Fig. 1). No difference was noted in the time course of the lethal reaction or the dose–response curve between the myeloma parent and either of the hybrid cell lines (Fig. 1).

TABLE 1. *Effect of dexamethasone on parental and hybrid cell lines*

Cell lines	Chromosome number Mode (range)*	Killing by dexamethasone	Cytoplasmic receptors† Dissociation constant (K_D) (nM)	Cytoplasmic receptors† Binding sites per cell
CL-4 (8-azaguanine-resistant)	60 (60–64)	+	2.6	6300
EL-4 (bromodeoxyuridine-resistant)	39 (38–41)	−	2.4	6600
N5 hybrid	86 (84–89)	+	2.7	8700
N6 hybrid	100 (99–103)	+	2.4	8900

* Data taken from ref. 21.

† Equilibrium dissociation constant (K_D) of receptor–dexamethasone complex and number of binding sites per cell were obtained from Fig. 3 based on yields of 22 and 18 pg cytosol protein per cell for CL-4 and EL-4, respectively, and a yield of 35 pg cytosol protein per cell for the hybrid cells. The values given are means of two independent experiments.

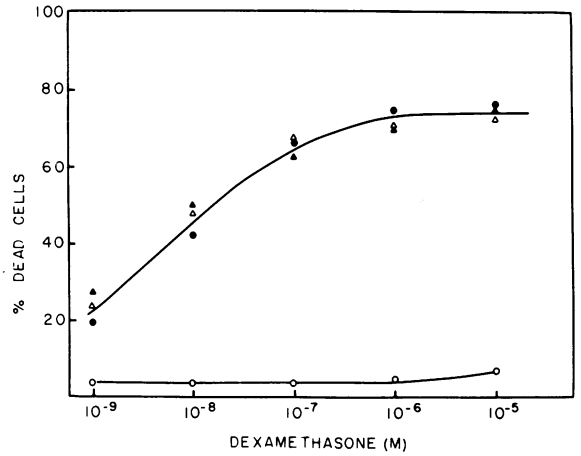

FIG. 1. Killing effect of dexamethasone. Cells were seeded at a density of 3×10^5/ml onto 5-cm Nunclon plastic petri dishes in 4 ml of medium to which dexamethasone (Sigma) has been added at various concentrations. After 48 hr at 37°, cell viability was determined by trypan blue exclusion in triplicate cultures. ●, CL-4; ○, EL-4; ▲, N5; △, N6.

Dexamethasone receptors in cytosols of parent and hybrid cells

As shown in Fig. 2, the cytosols of both parental cell lines as well as of the hybrid clones contained specific receptors for dexamethasone that became saturated at concentrations around 50 nM. When the binding data of Fig. 2 were plotted according to the Scatchard technique (26), linear relationships were obtained for all four cell lines studied (Fig. 3). The apparent dissociation constants (K_D) for the reaction: dexamethasone + receptor $\rightleftharpoons$ receptor–dexamethasone complex, were obtained from the plots of Fig. 3, and showed no significant differences between the parent and the hybrid cell lines (Table 1). The numbers of cytoplasmic binding sites for dexamethasone were about 6500 per cell for the parental cell lines and 8800 per cell for the hybrid clones (Table 1).

Table 2 summarizes competition experiments in which high concentrations of various unlabeled steroids were added to

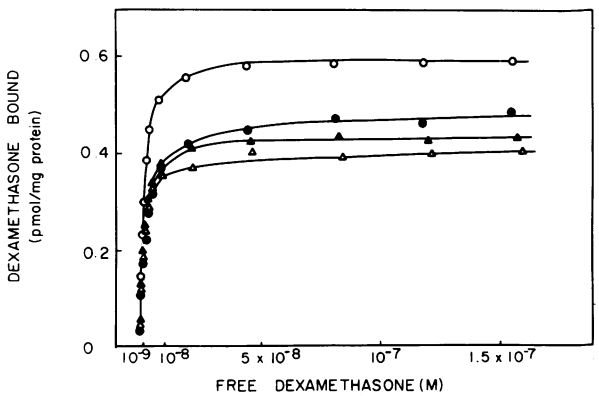

FIG. 2. Dexamethasone binding to cytoplasmic receptors. Cytosols from parent and hybrid cell lines were obtained as described in *Methods* and incubated with [^{3}H]dexamethasone at protein concentrations of 8 mg/ml. Specifically bound dexamethasone was determined (see *Methods*) and plotted as a function of the concentration of free steroid at equilibrium. The symbols are the same as in Fig. 1.

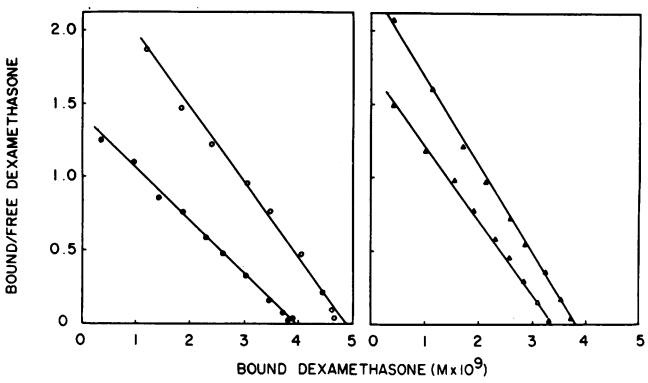

FIG. 3. Scatchard plot of the binding data shown in Fig. 2. The symbols are the same as in Fig. 1.

the binding assay with [^{3}H]dexamethasone. The results are compared with those obtained with rat hepatoma tissue culture cells. Optimal and suboptimal inducers of tyrosine transaminase (23) showed strong competition, compared to moderate competition by "anti-inducers." Steroids classified as "inactive" in the induction of tyrosine transaminase did not influence the binding. The pattern of competition observed with mouse myeloma and lymphoma cells and their hybrids was strikingly similar to that observed with the glucocorticoid receptors of rat hepatoma cells (Table 2). No differences were observed between the binding properties of the receptors of myeloma, lymphoma, and hybrid cells.

DISCUSSION

The present study demonstrates that a myeloma cell line can be killed by low concentrations (10 nM) of dexamethasone. Similar observations with two myeloma cell lines that produce IgA, MOPC-315 and J-558, show that this is not a unique property of the CL-4 line (our unpublished data and those of P. Ralph‡).

Our findings with CL-4 myeloma are consistent with earlier observations on lymphomas that cytoplasmic glucocorticoid receptors are required for the lethal effects of these steroids (17–19). The resistance of certain myelomas to steroids (9) may be due to the absence of specific cytoplasmic receptors as in the case of steroid-resistant lymphomas and fibroblasts (13, 19).

On the other hand, the present findings with the lymphoma line EL-4 demonstrate that while the possession of such receptors is necessary, it is not a sufficient condition for steroid-induced killing. Experiments in another glucocorticoid-responsive system, cultured rat hepatoma cells, have suggested that after binding of the hormone with its cytoplasmic receptor, the receptor–steroid complex associates with DNA-containing nuclear sites (27). Preliminary experiments with cell-free preparations from EL-4 have also indicated that its cytoplasmic receptor–dexamethasone complex can associate with the nucleus (unpublished data). It therefore seems likely that the resistance of EL-4 cells to steroids is at some step beyond the interaction of the receptor–steroid complex with the nucleus.

Experiments with thymocytes from intact animals have suggested that glucocorticoids stimulate the synthesis of macromolecules that somehow inhibit the uptake of various low molecular weight substances into the cell (28, 29). This inhibition is probably required for manifestation of the lethal effect and may not occur in the EL-4 lymphoma in response to the interaction of steroids with the cell.

It is not known whether the EL-4 line was derived from cells that were originally susceptible to glucocorticoids. Insensitivity may well have developed during more than 25 years of propagation of this lymphoma (25), and EL-4 cells may in fact have had steroid-sensitive progenitors, since they contain θ antigen, as do other thymus-derived lymphomas that are known to be sensitive to glucocorticoids (‡). Alternatively, EL-4 may be derived from a mature thymocyte, a cell that is resistant to glucocorticoids (30) but also has θ antigen.

Recent work has shown that a steroid-resistant variant of CL-4 myeloma can be selected that still contains cytoplasmic receptor molecules capable of association with the nucleus (unpublished data). These cells appear to be analogous to EL-4, which lends support to the argument that steroid-resistance in EL-4 cells could have arisen by a similar mechanism.

Insensitivity of EL-4 to dexamethasone might conceivably derive from the presence of an inhibitor of the reactions leading to the lethal response. This possibility cannot be easily reconciled with the demonstration that the hybrid clones derived from EL-4 and CL-4 cells are killed by dexamethasone (Fig. 1) (unless the putative inhibitor is for some reason not expressed in the hybrids). It seems more probable that EL-4 cells simply lack some component necessary for the lethal effect of glucocorticoids.

TABLE 2. *Competition of various steroids for dexamethasone binding to cytoplasmic receptors*

Steroid	[^{3}H]dexamethasone bound in the presence of unlabeled steroid (% of control)*					Biological activity in hepatoma cells‡
	CL-4	El-4	N5	N6	Hepatoma cells†	
Cortisol	2	1	2	2	0	Optimal inducer
Corticosterone	0	1	0	1	0	Optimal inducer
Dexamethasone	0	0	0	0	0	Optimal inducer
11β-Hydroxyprogesterone	1	1	1	1	0	Suboptimal inducer
17α-Hydroxyprogesterone	7	8	9	8	2	Suboptimal inducer
Progesterone	1	1	2	1	—	Suboptimal inducer
17α-Methyltestosterone	27	31	27	32	11	Anti-inducer
Testosterone	48	48	47	54	27	Anti-inducer
Epicortisol	108	99	98	102	99	Inactive
Androstenedione	89	82	91	93	79	Inactive

* The concentration of [^{3}H]dexamethasone in the binding assay was 10 nM, that of the unlabeled steroid 10 μM.

† Data taken from ref. 11.

‡ Classification of steroids according to their ability to induce tyrosine transaminase (23).

‡ Ralph, P. (1972) manuscript submitted to *J. Exp. Med.*

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