Characterization of Wild Type and Mutant Glucocorticoid Receptors from Rat Hepatoma and Mouse Lymphoma Cells*

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Using a combination of immunological blotting techniques and hormone affinity labeling, we have characterized the glucocorticoid receptors present in wild type and mutant rat hepatoma (HTC) and mouse thymoma (S49 and WEHI7) cells. Mutant HTC and WEHI7 cells of the receptorless phenotype, which contain greatly reduced amounts of glucocorticoid hormone binding activity, show parallel decreases in immunoreactive material using a monoclonal antibody raised against the rat liver glucocorticoid receptor. This indicates that these receptorless mutant cells harbor defects in either the production or accumulation of receptor protein. Quantitation of immunoreactivity and hormone binding activity present in wild type and mutant S49 cells indicates that these cells contain significantly more immunoreactive material than hormone binding activity. We conclude that S49 cells produce, in addition to their well characterized wild type or mutant receptors, a mutant receptor from a second allele which is of wild type size, is immunologically reactive, but is unable to bind hormone. The S49 mutant cell line nt' (nuclear transfer increase) contains a glucocorticoid receptor which has a molecular weight of 40,000, while the wild type receptor has a molecular weight of 94,000. Affinity labeling of glucocorticoid receptors in nt' cells with [3H]dexamethasone mesylate indicates that nt' cells do not contain wild type sized precursor molecules which bind hormone, nor do they contain immunoreactive fragments of a molecular mass smaller than 94 kDa. It is proposed that the 40-kDa nt' receptor is produced as a truncated protein most likely resulting from a nonsense mutation or from a truncated messenger RNA.

Steroid hormones mediate physiological responses in target cells via an interaction with specific, high affinity receptors. Binding of hormone to these receptors results in a structural alteration in the receptor protein, allowing the steroid-receptor complex to bind tightly to acceptor sites within the nucleus (for reviews see Refs. 1 and 2). The association of steroid-receptor complexes with specific DNA sequences in the 5' flanking regions of target genes such as those encoding the mouse mammary tumor virus (MMTV) (3, 4), chicken lysozyme (5), and human metallothionein-IIa (6) appears to be important for transcriptional activation of their respective promoters.

We have previously described the isolation, using fluorescence-activated cell sorting, of variant rat hepatoma (HTC) cells which are unresponsive to glucocorticoids (7). These variants have less than 10% of wild type hormone binding activity and are incapable of inducing any of their characteristic glucocorticoid-responsive genes such as MMTV, tyrosine amino transferase, glutamine synthetase, Belt I (8), and α1-acid glycoprotein (9). This coordinate loss of responsiveness suggests that a single class of glucocorticoid receptor mediates the induction of the glucocorticoid domain (8, 10).

The selection of lymphoma cell variants resistant to the cytolitic effects of glucocorticoid hormones (11, 12) has been useful in the characterization of the structure and function of glucocorticoid receptors. Three classes of lymphoma cell variants have been characterized. The r- (receptorless) variant contains very little and in some cases no specific steroid binding (13), while the nt- (nuclear transfer-deficient) variant contains a receptor which binds hormone but subsequently has reduced affinity for DNA (14, 15). The nt' (nuclear transfer increased) variant receptor has an elevated affinity for nuclei and DNA after binding hormone (15). Biochemical analysis of the glucocorticoid receptors present in these variant cell lines (16, 17) and, in particular, partial proteolysis of wild type and mutant receptors (18-20) has led to the following model for the structure of the glucocorticoid receptor (19, 20).

The receptor is a 92,000-94,000-dalton polypeptide containing distinct functional domains in which the hormone and DNA-binding regions reside on an approximately 40,000-dalton fragment that can be generated by mild chymotrypsin treatment of wild type receptor. This 40,000-dalton fragment appears to be similar to the truncated form of the wild type receptor present in the nt' variant (17). The nt' variant is similar in size to the wild type receptor, but contains a defect in the DNA-binding site (14). The remainder of the molecule harbors antigenic sites of the receptor and appears to have a role in modulating DNA binding activity (19, 20).

In this study, we have used immunological techniques and hormone affinity labeling to characterize wild type and mutant glucocorticoid receptors in rat hepatoma and mouse lymphoma cells. We find that unresponsive rat hepatoma cells and mouse WEHI7 lymphoma cells are depleted of immunologically detectable glucocorticoid receptor. Our results also indicate that in addition to the well characterized

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The abbreviations used are: MMTV, mouse mammary tumor virus; nt', nuclear transfer-deficient; nt', nuclear transfer increased; r-, receptorless; NaDodSO4, sodium dodecyl sulfate; PBS, phosphate buffered saline; BSA, bovine serum albumin.

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wild type, nt', and nt- receptors. S49 mouse lymphoma cells contain a receptor which is immunologically reactive but has no hormone binding activity. We also present evidence suggesting that the nt' receptor is not produced as a large precursor molecule of the same size as the wild type receptor. In addition, it appears that the wild type glucocorticoid receptor exists in two forms which run at slightly different molecular weights on NaDodSO4 gels; both forms are immunologically reactive and bind hormone.

MATERIALS AND METHODS

Cells and Media—MSC1 is a glucocorticoid-responsive subclone of the MMTV-infected HTC cell line M1.19. M1.19 contains 8–16 MMTV proviral copies per genome, at least some of which are inducible by dexamethasone (21). MSN.53 is a nonresponsive clone isolated through five rounds of selection on a fluorescence-activated cell sorter for low induction of the MMTV glycoprotein gp52 (7). W7.2 is a subclone of the mouse T-lymphosarcoma cell line, WEHI7 (22). ADR38 and ADR6 are glucocorticoid-resistant clones selected from an ethyl methanesulfonate-nitrogenated population of W7.2 cells in 1 μM dexamethasone (23). ADR5189D is a glucocorticoid-resistant cell line derived from MMTV-infected ADR6 cells. ADR56189D cells have no detectable glucocorticoid-binding component (38). Wild type cell lines S49.JN and 49.T.B4.1A.2 (denoted here as S49.A2) are steroid-sensitive subclones of the lymphoma line S49.1A.7R (r-) are steroid-resistant variants originally selected by Sibley and Tomkins (12). HTC cells were grown as monolayers in Dulbecco's modified Eagles medium (Irvine Scientific) supplemented with 10% fetal bovine serum (FBS) and 1 mM sodium pyruvate (GIBCO) in a 5% CO2 atmosphere at 37 °C. HTC cells were grown as monolayers in RPMI 1640 medium (Irvine Scientific) supplemented with 10% FBS and 1 M sodium pyruvate (GIBCO) in a 5% CO2 atmosphere at 37 °C. HTC cells were grown in suspension in RPMI 1640 medium (Irvine Scientific) supplemented with 10% FBS and 1 M sodium pyruvate (GIBCO) in a 5% CO2 atmosphere at 37 °C.

Whole Cell Binding Assays—Glucocorticoid receptor levels were assayed in whole cells as previously described (16). Briefly, 1 × 107 cells were harvested and resuspended in 1 ml of serum-free RPMI. To this was added 0.5 ml of serum-free RPMI containing 2 × 10-4 M [*H]dexamethasone (Amersham, 72 Ci/mmol). Nonspecific binding was assayed by adding 2 × 10-4 M unlabeled dexamethasone to the incubations. Cells were incubated with hormone at 37 °C for 40 min, spun down, and washed three times by resuspension and resedimentation in ice-cold phosphate buffered saline (PBS) containing CaCl2 and MgCl2/6H2O at 0.1 g/liter each. Radioactivity in whole cell pellets was assayed in Aqueous Counting Scintillant (Amersham) at 37% efficiency.

Preparation of Cytosols—HTC cells were harvested using PBS containing 2.5 mM EDTA, washed once with PBS, and cell pellets were quickly frozen in liquid nitrogen. Lymphoma cells were washed with PBS and cells pellets frozen as above. Frozen cells were stored at −70 °C until used. Frozen cell pellets, 1 × 107 lymphoma cells or 2 × 108 HTC cells, were thawed and homogenized in a Dounce homogenizer in 250 μl of buffer (20 mM sodium phosphate, pH 7.4, 1 mM EDTA, 50 mM NaCl, 2 mM β-mercaptoethanol, and 10% glycerol) and centrifuged 5 min at 10,000 × g. The supernatant was made 20 mM in sodium molybdate when it was to be used for affinity labeling, in which case it was used immediately. Otherwise, supernatants were stored frozen at −70 °C. Protein concentrations were determined by the method of Bradford (24) using Bradford reagent (Bio-Rad) with bovine γ-globulin as standard.

Immunoblotting—Aliquots of cytosols containing 150–200 μg of total protein were heated to 100 °C for 2.5 min in 62 mM Tris-HCl, pH 6.7, 8% glycerol, 2% β-mercaptoethanol, containing a trace of b-mercaptoethanol as a mediator. Standard molecular weight marker proteins used were myosin (200,000), β-galactosidase (116,250), phosphorylase b (92,500), bovine serum albumin (66,200), and ovalbumin (45,000). Electrophoresis in NaDodSO4 containing polyacrylamide gels was done according to Laemmli (25) using a 7.5% separating gel. Electrophoresis in NaDodSO4 containing polyacrylamide gels was done according to Laemmli (25) using a 7.5% separating gel. Proteins were separated on NaDodSO4-polyacrylamide gels as described above. Typically, these aliquots contained from 2500 to 9000 cpm of protein-bound [*H]dexamethasone. After Coomassie Blue staining and fixation in 10% (v/v) acetic acid, 10% (v/v) isopropl alcohol, gels were soaked for 30 min in water, then for 30 min in 1 M sodium salicylate (Mallinkrodt), and then dried and submitted to fluorography with Kodak XAR5 film at −70 °C for 5–10 days. Scanning densitometry was performed on an EC Apparatus Corp. densitometer.

In Vivo Affinity Labeling—Approximately 2.5 × 108 cells were harvested, washed once with PBS, and resuspended on 0.5 ml of serum-free RPMI containing 2 × 10-7 M [*H]dexamethasone mesylate. These were incubated at 37 °C for 25 min, then harvested by low speed centrifugation, and washed once with ice-cold PBS containing CaCl2 and MgCl2/6H2O, 0.1 g/liter each. Cells were then sonicated on ice in 200 μl of buffer (20 mM sodium phosphate, pH 7.4, 1 mM EDTA, 50 mM NaCl, 2 mM β-mercaptoethanol, and 10% glycerol) using a Heat Systems-Ultrasonic, Inc. sonicator, 3 × 10-s pulses with 23-s cooling in between. Insoluble debris was spun out (5 min at 10,000 × g), and the supernatant was stored at −70 °C. Aliquots containing 150–200 μg of protein were analyzed as described under affinity labeling.

RESULTS

Characterization of HTC and WEHI7 r- Variants—We have previously isolated glucocorticoid-unresponsive clones of the MMTV-infected cell line M1.19 using a fluorescence-activated cell sorter (7). Such cells (e.g. MSN 5.3) contain less than 10% of the glucocorticoid receptor present in wild type cells as determined by binding of [*H]dexamethasone. To test the possibility that MSN 5.3 cells produce a receptor protein that is defective in hormone binding, we have assayed extracts of these cells with a monoclonal antibody that recognizes both mouse and rat glucocorticoid receptor. Filters were incubated for 16 h at 4 °C with BuGR1 (26), a monoclonal antibody. Filters were then washed twice in T/S containing 2% BSA. After washing two times (5 min each) with T/S/N, the filters were then reincubated in T/S containing 2% BSA for 30 min. After this second blocking incubation, filters were processed in one of three ways. They were incubated at room temperature for 2 h either in 361 protein A (New England Nuclear, 70–100 μCi/μg) diluted to 200,000 cpm/ml in T/S/N, 2% BSA, or in horseradish peroxidase-conjugated goat anti-mouse IgG (Bio-Rad) diluted 1:1000 in T/S/N, 2% BSA, or in biotinylated horse anti-mouse IgG (Vector Labs) diluted to 2 μg/ml in T/S/N, 2% BSA. In the case of the biotinylated secondary antibody, after washing two times (5 min each) with T/S/N and either dried and autoradiographed or developed in the peroxidase substrate 3,3′-diaminobenzidine (Sigma), cell pellets were stained with alkaline phosphatase to detect BuGR1. After Coomassie Blue staining and fixation in 10% (v/v) acetic acid, 10% (v/v) isopropl alcohol, gels were soaked for 30 min in water, then for 30 min in 1 M sodium salicylate (Mallinkrodt), and then dried and submitted to fluorography with Kodak XAR5 film at −70 °C for 5–10 days. Scanning densitometry was performed on an EC Apparatus Corp. densitometer.
Glucocorticoid Receptor Mutants

**TABLE 1**

**Steroid and antibody binding of wild type and mutant receptors**

| Cell type          | I, specific steroid binding relative to W7.2 | II, immuno-reactive material relative to W7.2 | III, \[^{3}H\]dexamethasone mesylate-labeled receptor relative to W7.2 |
|--------------------|---------------------------------------------|---------------------------------------------|------------------------------------------------|
| HTC                |                                             |                                             |                                                |
| Wild type (MSC1')  | 100                                         | 100                                         |                                                |
| r' (MSCN5.3)       | ≤10% of MSC1'                               | 12\(^b\)                                    |                                                |
| WEHI7              |                                             |                                             |                                                |
| r' (ADR6.M189D)    | 0\(^a\)                                     | 0\(^b\)                                     |                                                |
| r' (ADR38)         | 22 ± 2\(^ \pm \)                            | 23\(^b\)                                    |                                                |
| Wild type (W7.2)   | 100                                         | 100                                         | 100                                            |
| S49                |                                             |                                             |                                                |
| Wild type (S49.JN) | 81 ± 7 (4)                                   | 167 ± 5 (3)                                 | 50                                             |
| Wild type (S49.A2) | 58 ± 2 (3)                                   | 133 ± 6 (3)                                 | 43                                             |
| nt' (S49.1A.55R)   | 131 ± 16 (3)                                | 86 ± 7 (3)                                  | 121                                            |
| nt' (S49.1A.22R)   | 29 ± 1 (3)                                   | 183 ± 25 (3)                                | 51                                             |
| r' (S49.1A.7R)     | 7.5 ± 2 (2)                                  | 41 ± 3 (3)                                  | <5                                             |

\(^a\) Data on HTC cells is relative to MSC1 not W7.2; quantitated by scanning densitometry of immunoblots.

\(^b\) Results of a single experiment.

\(^c\) Data from Grove et al. (7).

\(^d\) Ref. 38.

\(^e\) Data from Danielsen and Stallcup (23).

**Fig. 1 (left). Immunoblot of HTC cell cytosols electrophoresed on a NaDodSO\(_4\) gel.** Cytosols were prepared and analyzed as described under "Materials and Methods." \[^{125}I\]protein A was used to detect the monoclonal antibody. The glucocorticoid-unresponsive cell line, MSN5.3, contains less than 10% of the glucocorticoid receptor present in the responsive cell line, MSC1. Molecular weight markers are myosin (200,000), \(\beta\)-galactosidase (116,250), phosphorylase b (92,500), bovine serum albumin (66,200), and ovalbumin (45,000).

**Fig. 2 (right). Horseradish peroxidase-stained immunoblot of WEHI7 cell cytosols.** Cytosols were analyzed as described under "Materials and Methods." Detection of the monoclonal antibody was accomplished using a biotinylated horse anti-mouse IgG and subsequent incubation with avidin-biotin-peroxidase complex and development in diaminobenzidine solution. Bands detected at higher and lower molecular weights are due to nonspecific binding of the second antibody as they are present even when the monoclonal antibody is omitted (data not shown). Lane 1 (ADR6.M189D), lane 2 (ADR38), lane 3 (W7.2). Marker proteins are those used in Fig. 1.

Table 1 shows the results of steroid and antibody binding experiments for wild type and mutant receptors. The top row of the table shows the cell type being tested. The second column, labeled I, presents the specific steroid binding relative to the wild type W7.2. The third column, labeled II, shows the immuno-reactive material relative to the wild type W7.2. The fourth column, labeled III, shows the \[^{3}H\]dexamethasone mesylate-labeled receptor relative to the wild type W7.2.

**Fig. 2 shows an immunological analysis of extracts from wild type and r' cells derived from the W7.2 lymphoma cell line by sequential ethyl methanesulfonate mutagenesis and growth in 1 \(\mu\)M dexamethasone (23).** Danielsen and Stallcup (23) have reported that the r' cells contain from one-fourth (ADR38) to one-half (ADR6) the number of receptors as the sensitive parental cells (see Table I). Clone ADR6, after infection with mouse mammary tumor virus and reselection in dexamethasone, yields clones (e.g., ADR6.M189D) that contain no detectable hormone binding activity. Our results show a very close parallel between the hormone binding activity of such cells and the presence of immunologically reactive receptor protein (Table I). Clone ADR38 contains reduced levels of a 94-kDa receptor protein as compared to the parental W7.2 cells, and no immunologically reactive receptor can be detected in the ADR6.M189D cells (Fig. 2). Thus, as with the rat hepatoma variants which were selected by completely different protocols, defects in hormone binding activity of W7.2 mutants appear to be related to decreased production or stability of receptor rather than production of receptor protein with a defect in hormone binding activity.

**Receptor Defects in S49 Cells** — The vast majority of glucocorticoid-resistant S49 cells are of the r' phenotype in that they lack wild type levels of hormone binding activity (13). Characterization of such cells with the anti-receptor monoclonal antibody, however, reveals that they contain significant amounts of immunologically detectable receptor protein (Fig. 3, lane 5). When compared to its wild type parent, S49.A2 (Fig. 3, lane 2), the r' clone S49.1A.7R contains approximately one-third the amount of immunologically cross-reactive receptor yet little more than 10% the level of hormone binding activity (Table I). In addition, two different clones of wild type S49 cells contain approximately twice as much immunoreactive receptor per unit of hormone binding activity as compared to W7.2 cells (Table I). These results strongly suggest that all clones of S49 cells express a mutant form of the glucocorticoid receptor which is unable to bind hormone; in r' cells, it appears that the second functional allele has been inactivated.

As described earlier, S49 nt' cells contain normal or in some cases higher levels of glucocorticoid binding activity relative to wild type S49 cells.
Affinity Labeling—Simons and Thompson (27) have used the "Materials and Methods." Peroxidase-coupled anti-mouse IgG and development antibody. To wild type S49 cells (see Table I). The receptor in these cells has been reported to behave as a 40-kDa protein that contains both hormone and DNA binding activities (17, 20). When extracts of such cells are assayed by immunoblotting, the only detectable receptor protein migrates at approximately 94 kDa (Fig. 3, lane 3) and appears indistinguishable from receptors present in wild type cells (Fig. 3, lanes 1 and 2); the amount of detectable protein, however, is approximately 50-60% of that in S49 cells (Table I). This result is consistent with the presence of a nonhormone-binding form of receptor that is of wild type size. We have not detected the presence of a smaller form of the glucocorticoid receptor immunologically, although a 40-kDa form is easily detectable by hormone affinity labeling (see below).

Characterization of Hormone-binding Forms of Receptor by Affinity Labeling—Simons and Thompson (27) have used the α-ketonesylate derivative of dexamethasone to affinity label glucocorticoid receptors from rat hepatoma cells. We have used this reagent to both quantify and estimate the size of the glucocorticoid receptor in W7.2, S49, and various mutant cell lines. Fig. 4A shows an autoradiogram of [3H]dexamethasone mesylate-labeled cytosol proteins analyzed by Na-DodSO₄-gel electrophoresis. A prominent band appears at approximately 94 kDa in cytosols from wild type and nt- cells (lanes 1, 2, 5, and 6) that is missing in the r- S49 cells (lane 4). By scanning densitometry of these bands, the relative amounts of receptor present in each of the cell lines can be determined; the results summarized in Table I, column 3, document that there is very good agreement between this assay and a standard hormone binding assay. Since W7.2 cells are smaller than S49 cells, one would expect to see a lower number of receptors in S49 relative to W7.2 when the receptor levels are calculated on the basis of protein content rather than on a per cell basis. Except for the nt- line, this is in fact observed (compare Table I, column 1 to column 3). The discrepancy in the values found for the nt- line is most likely due to a subtle alteration in the binding site which increases the equilibrium dissociation constant Kd for dexamethasone, except when the hormone can be covalently attached. In support of this point, Spindler-Barth and Gehring (31) have reported that the nt- clone S49.1A.22R had a Kd for dexamethasone which is 3-fold higher than that of the wild type and nt- clones tested.

In the case of nt- cells, dexamethasone mesylate predominantly labels a 40-kDa species of receptor (Fig. 4, lane 3). No labeling of a 90-95-kDa protein can be detected. Incubation of the extracts with dexamethasone mesylate in the presence of a large excess of unlabeled dexamethasone abrogates the labeling of the 94-kDa receptor in wild type cells and of the 40-kDa form in the nt- cells (Fig. 5, panel A). Additional bands that are nonspecifically labeled by the dexamethasone mesylate (e.g. the band at about 70 kDa) cannot be competed by dexamethasone.

Fig. 4B shows an immunoblot of the dexamethasone mesylate-labeled cytosols shown in Fig. 4A. Analysis of the relative amount of immunoreactive protein in these cells yields results identical to those presented in Table I, column 2. Thus, the labeling procedure itself does not affect the ability of the monoclonal antibody to recognize the receptor. By comparison of these analyses, it is clear that both r- and nt- cells contain significant amounts of immunoreactive receptor of approximately 94 kDa that is incapable of binding hormone.

Origin of the 40-kDa nt- Receptor—The analyses presented above indicate that no hormone-binding receptor of 94 kDa can be found in nt- cells. Since the previous results were
obtained using cytosols prepared from each of the cell lines, it seemed conceivable that we might be missing a precursor present in nuclei or that in the process of preparing the extracts we might be degrading a larger precursor. We have tried to address these issues by labeling the receptors in nt' cells as well as wild type cells in vivo. Fig. 5, panel B, shows a NaDodSO₄ gel of the proteins labeled in intact cells. Although there is a significant increase in nonspecific labeling of proteins, it is clear that a prominent band at about 94 kDa is present in each band when affinity labeled cytosols are analyzed in the same way. Bands are cut out of the immunoblot and assayed for radioactivity in aqueous counting scintillant.

r- cell lines also contain these two immunoreactive species at 94 kDa; however, as discussed above, they are incapable of binding hormone. Although we cannot rule out that the lower species is a proteolytic fragment, it is clear that the two species are not from two different alleles since nt' cells contain both.

**DISCUSSION**

Using a monoclonal antibody and the affinity label dexamethasone mesylate, we have characterized glucocorticoid receptor mutants of rat hepatoma (HTC) cells and mouse lymphoma cells (S49 and WEHI7). Both HTC and WEHI7 r- mutants show parallel decreases in hormone binding activity and immunoreactive material (Table 1). We conclude that these cells harbor defects in either the production or accumulation of receptor protein. A defect in the production of functional receptor protein could be due to a decreased amount of receptor messenger RNA, a possibility which cannot be tested at this time, or alternatively to a truncated messenger RNA or nonsense mutation resulting in the production of a smaller nonfunctional protein. The residual receptor present in the r- cells is of identical size to that of the wild type cells; furthermore, no smaller receptor species can be detected by hormone binding or immunoblotting. Therefore, although it cannot be ruled out, the existence of a smaller nonfunctional receptor in these r- cells seems unlikely. Numerous reports have shown, however, that proteins such as β-galactosidase (32), hypoxanthine-guanine phosphoribosyltransferase (33), and hemoglobin (34) which contain slight structural alterations due to missense mutations, are rapidly degraded and do not accumulate within cells to the extent that the wild type protein does. It is possible that the HTC and WEHI7 r- mutants also arise through missense mutations which cause the receptor protein to be unstable. Eventual analysis of the receptor messenger RNA and gene should resolve this point.

The S49 nt- receptor appears to contain a defect in the DNA-binding site (14). Our immunological and affinity labeling experiments are consistent with previous observations indicating that this receptor binds hormone, is very similar in size to the wild type receptor (18, 20), and reacts with monoclonal antibodies to the rat liver receptor (35). The difference in the amount of hormone binding seen in the nt- cells when assayed using the affinity reagent, dexamethasone mesylate, as opposed to dexamethasone most likely reflects a subtle alteration in the hormone-binding site which increases the $K_d$ for the noncovalently bound hormone.
Comparison of hormone binding activity and immunoreactivity of S49 wild type and variant cells lines relative to W7.2 cells (Table 1) reveals that there is significantly more immunoreactive material than would be expected from the hormone binding data. The only immunoreactive receptor present in nt' cells is a 94-kDa protein which is devoid of hormone binding activity (Fig. 4). These results indicate that all S49 cells contain a mutant form of the receptor which does not bind hormone. A variety of biochemical and genetic data on S49 and WEHI7 cells have been presented by Bourgeois and Newby (11). They suggest that wild type S49 cells are functionally haploid while WEHI7 cells are functionally diploid for the glucocorticoid receptor. Our results explain the genetic behavior of these two cell types by showing that S49 cells contain the nonfunctional product of a second receptor allele distinct from the product of the first allele detected by hormone binding studies. In a recent report, Westphal et al. (35) have also arrived at this conclusion for S49 cells using slightly different techniques.

The receptor has a molecular mass of approximately 40 kDa (18, 20, Fig. 4). This receptor form could arise by several different mechanisms. A nonsense mutation or a deletion in the structural gene could lead to the production of a truncated receptor protein. Another possibility is that of a point mutation causing the production of a receptor which is unstable. Partial proteolysis of wild type receptors with chymotrypsin yields a receptor which has a similar size and hormone and DNA binding affinity to that of the nt' receptor (18). Carlstedt-Duke et al. (19) have been able to detect immunologically a chymotryptic fragment derived from wild type receptor which does not contain the hormone or DNA-binding sites. If the 40-kDa nt' receptor arises from a larger unstable precursor, one might expect to be able to detect such an immunoreactive receptor fragment. Furthermore, this larger precursor molecule would presumably bind hormone and should be detectable using an affinity label. Our results do not show the presence of either a large hormone-binding receptor (Figs. 4 and 5) or a small immunoreactive fragment (Figs. 3 and 4) in nt' cells. Although these findings do not rule out the possibility of an extremely labile 94-kDa or smaller precursor molecule, it seems most likely that the 40-kDa nt' receptor is produced as a truncated protein resulting from a nonsense mutation or from a truncated messenger RNA.

Recently, Gruol et al. (36) have reported that nuclei of nt' cells contain a hormone-binding glucocorticoid receptor of similar size to that of wild type cells; however, nt' cytosol contains only the smaller 40-kDa nt' receptor protein. Their data was obtained using cells labeled in vivo as is our data (Fig. 5, panel B). We do not at this time understand the basis for this discrepancy.

In both wild type and mutant lymphoma cells, the 94-kDa receptor species appears to migrate as a doublet on NaDodSO4 gels. Both species of the doublet are immunoreactive; however, only those from wild type and nt' cells are capable of binding hormone. Housley and Pratt (37) have detected two species of glucocorticoid receptor of approximately 92 kDa in glucocorticoid-sensitive L cells, both of which are phosphorylated and bind hormone. Although we cannot at this time say what the relationship between the two species of the doublet is, both bind hormone equally well, at least in wild type and nt' cells, and they appear not to be the product of two separate alleles since nt' cells contain both. A more complete understanding of the structural and functional domains of wild type and mutant glucocorticoid receptors will depend on characterizing their amino acid sequences.

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