The EMBO Journal Vol.1 No.11 pp.1467 – 1471, 1982

Monoclonal antibodies to the rat liver glucocorticoid receptor

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Communicated by P. Karlson
Received on 11 October 1982

Monoclonal antibodies against the 90 000 mol. wt. form of the activated rat liver glucocorticoid receptor were generated from mice immunized with a partially purified receptor preparation. The screening assay was based on the precipitation of liver cytosol, labelled with [^{3}H]triamcinolone acetonide, with monoclonal antibodies bound to immobilized rabbit anti-mouse IgG. Out of 102 hybridomas obtained, 76 produced immunoglobulin and eight of them were found to react with the receptor molecule. Only one of the positive clones secreted IgG whereas the other seven produced IgM. The complexes of receptor and antibodies were identified by sucrose density gradient centrifugation. All seven monoclonal antibodies tested reacted with the 90 000 mol. wt. form of the receptor but not with the 40 000 mol. wt. form that contains the steroid and DNA binding domains. None of the monoclonal antibodies interfered with the binding of the receptor to DNA cellulose, thus suggesting that the antigenic determinants are located in a region of the receptor that is not directly implicated in either steroid binding or DNA binding. These antigenic determinants were common to glucocorticoid receptors from several tissues of the rat, whereas glucocorticoid receptors from other species react only with some of the antibodies.

Key words: steroid hormone receptors/monoclonal antibodies/DNA binding

Introduction

Steroid hormone receptors are proteins which bind the steroid specifically, and non-covalently. According to the generally accepted model of steroid hormone action, the hormone-receptor complex is then translocated to the cell nucleus and interacts with the chromatin to elicit the specific hormone responses of the cell. In this model the hormone acts as a trigger for the receptor to become operative. Thus, considerable effort has been made to elucidate the structure of these receptors. Antibodies directed against the receptors are a useful tool to characterize these molecules, to study relationships between receptors of different species, and to purify receptors by immunoaffinity chromatography. As to the glucocorticoid receptor, several workers have described the preparation and application of polyclonal antibodies. (Govindan, 1979; Eisen, 1980; Okret *et al.*, 1981). These studies are limited by the heterogeneity of the antibody preparations. In this paper we describe the preparation and characterization of monoclonal antibodies against the glucocorticoid receptor of rat liver. Because of their clonal origin, these antibodies should serve as fine probes for the structural and functional analysis of the receptor molecule.

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Results

Generation and screening of the antibodies

The receptor preparation used for immunization was obtained from fresh rat livers as described by Wrange *et al.* (1979). Figure 1 shows the analysis of such a preparation on a SDS-polyacrylamide gel. The receptor represents ~30% of the total protein in the final preparation, and was identified by photoaffinity labelling (Gronemeyer and Pongs, 1980; Westphal *et al.*, 1981). Monoclonal antibodies to the glucocorticoid receptor of rat liver were generated by the somatic cell hybridization technique (Köhler and Milstein, 1975). The hybridomas presented here were derived from one successful fusion (1GR) of mouse myeloma cells (X63-Ag8.653) and splenic lymphocytes from BALB/c mice immunized with partially purified native receptor from rat liver cytosol. After HAT selection, 85% (102/120) of the initial cultures contained hybrids. These were tested for mouse immunoglobulin synthesis by enzyme-linked immunoadsorbent assay (ELISA). Since the myeloma cell line used lacked the ability to produce its own immunoglobulin, all Ig-positive cultures were expected to secrete monoclonal antibodies. Out of 102 hybridomas obtained, 76 produced immunoglobulin (~75%).

Subsequently, mouse Ig-positive supernatants from the cultures were screened for binding to the glucocorticoid receptor by immunoprecipitation of the [^{3}H]steroid-receptor complex. The method used was a double antibody technique: rabbit anti-mouse IgG-Sepharose (RAM-Sepharose) was added to the supernatants of the hybridoma cultures and in-

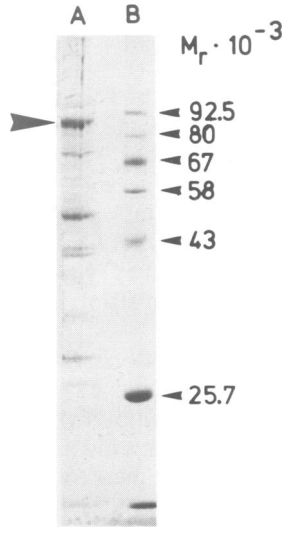

Fig. 1. SDS-polyacrylamide gel electrophoresis of the partially purified glucocorticoid receptor (**lane A**). The arrow on the left denotes the position of the receptor. **Lane B** shows the marker proteins used (from top to bottom): phosphorylase b, transferrin, bovine serum albumin, catalase, ovalbumin, and chymotrypsinogen A.

cubated for 2 h. The RAM-Sepharose was centrifuged off, washed with phosphate-buffered saline (PBS) and incubated with rat liver cytosol glucocorticoid receptor labelled with [^{3}H]triamcinolone acetonide. Then the RAM-Sepharose was again washed with PBS and the bound radioactivity was measured. This method depended on the detection of the steroid and therefore the screening detected only antibodies which were neither directed against the steroid binding site nor influenced the steroid binding indirectly.

Eight hybridomas were found to secrete monoclonal antibodies reacting with the receptor molecule (~11% of immunoglobulin-producing hybrids). These cultures were cloned by limiting dilution and a representative clone eliciting anti-receptor activity was selected for each hybridoma. The immunoglobulin heavy chain isotype of the eight antibodies was determined (Table I). Seven antibodies were found to be of the IgM type and one was an IgG. The ability of the different supernatants to bind the glucocorticoid receptor is shown in Table I. Clones 25, 49, 57 and 98 were chosen for further characterization. Larger quantities of anti-receptor monoclonal antibodies were produced in ascitic fluid of BALB/c mice. Milligram amounts of desired antibody were obtained per ml of ascitic fluid.

Sedimentation of antibody-receptor complexes

The formation of antibody-receptor complexes was analysed in sucrose gradients. To saturate the receptor, a 10- to 20-fold excess of antibody was incubated with cytosol receptor for 2–3 h and then layered on a 5–20% sucrose gradient. After 3 h at 360 000 *g* the sedimentation constant of the IgM-receptor complexes was 19S as for IgM molecules alone. About 40–50% of the input receptor sediments free of antibody with a sedimentation constant of ~4S. This is consistent with the finding of the titration curves (see below). In Figure 2A the complex of antibody from clone 25 with the receptor is shown. A similar profile was found with the antibody from clone 98, whereas the clone 57 antibody yields a broader peak.

A longer centrifugation in the same sucrose gradient separates the clone 49 IgG-receptor complex from free receptor (Figure 1B). Again, not all receptor is complexed despite a 20-fold antibody excess.

Titration of the antibodies

To determine the affinity of the monoclonal antibodies for the glucocorticoid receptor, increasing amounts of antibodies were added to 0.1 pmol cytosol receptor. After incubation overnight at 4°C, the bound receptor was determined with RAM-Sepharose as described in Materials and methods. The apparent dissociation constants K_d of the antibody-receptor complexes were determined by Scatchard plot analysis (Figure 3 inset). As shown in Table I, they differ by two orders of magnitude.

The IgG antibody of clone 49 binds the receptor quantitatively (70–100%); whereas only 45–65% of the receptor could be bound by the IgM antibodies of clones 25, 57, and 98 (Figure 3). The reason for this incomplete binding remains unclear. One possibility is that there are immunologically different populations of receptor molecules. If this holds true, mixing of different antibodies should increase the percentage of receptor that can be precipitated by RAM-Sepharose. However, when the three IgM antibodies were mixed and incubated with cytosol no significant increase in receptor binding was found (Table II). Therefore, immunological heterogeneity does not seem to be responsible for the incomplete binding of the receptor to the IgM monoclonal antibodies.

Table I. Binding of serum from the immunized mice and antibodies from eight hybridoma culture supernatants to the glucocorticoid receptor

Sample		% Receptor binding[a]	K_d[b] (mol/l) x 10^9	Heavy chain isotype[c]
Mouse antiserum	0.050 ml	15.2		
	0.005	5.1		
	0.001	2.6		
Clone number 1 GR	25	33.5	3.7	IgM
	30	15.9		IgM
	49	37.8	6.7	IgG_1
	57	38.0	0.5	IgM
	67	16.4		IgM
	86	29.0		IgM
	91	28.9		IgM
	98	18.0	77	IgM
Culture medium		1.7		

[a]Binding measured with the immunoprecipitation test (screening test as described in Materials and methods).
[b]The dissociation constants K_d were evaluated from Scatchard plot analysis (see Figure 3).
[c]The heavy chain isotype of monoclonal antibodies was determined by double antibody sandwich ELISA.

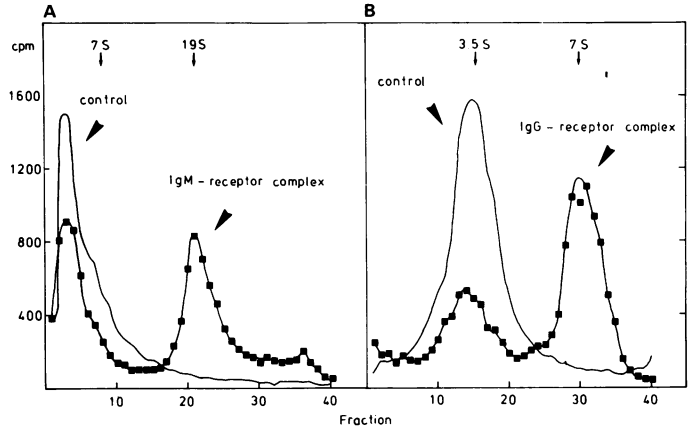

Fig. 2. Sedimentation profile of the antibody receptor complexes. 50 µl cytosol labelled with [^{3}H]triamcinolone acetonide (0.5 pmol receptor) was incubated with a 10- to 20-fold excess of antibody for 2–3 h and then layered on a 5–20% sucrose gradient (in PBS containing 0.3 M NaCl). **A**: —■——■ IgM (from clone 25) with receptor. ——— control: ascitic fluid of an irrelevant hybridoma secreting monoclonal IgM. Sedimentation markers were: human IgG (7S) and human IgM (19S), centrifugation for 3 h at 360 000 *g*. **B**: —■——■— IgG (from clone 49) with receptor. ——— control: human polyclonal IgG. Sedimentation markers were ovalbumin (3.5S) and human polyclonal IgG (7S), centrifugation for 17 h at 250 000 *g*.

Influence on DNA binding

It was of interest to test whether the binding of the antibody affects the DNA-binding capacity of the receptor. Incubation of cytosolic receptor with saturating amounts of antibody (as determined from Figure 3) does not influence the binding of the receptor to DNA-cellulose (Table II). The DNA binding of the receptor was in all cases ~60%, although 40–80% of the receptor was complexed with anti-

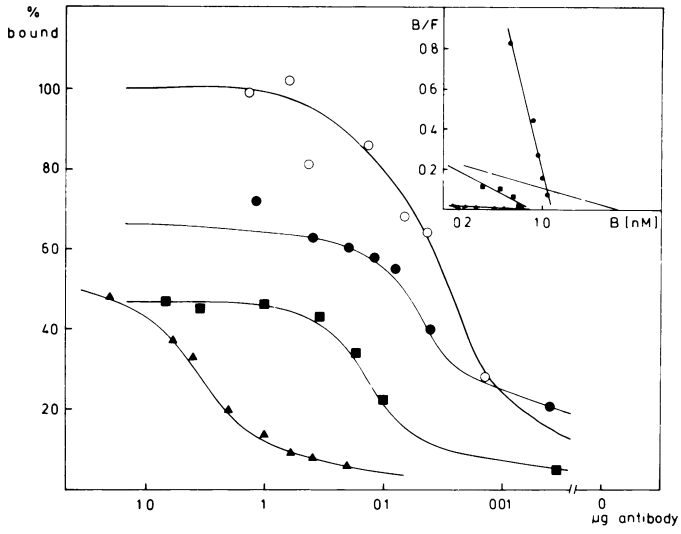

Fig. 3. Titration of monoclonal antibodies from ascitic fluid against cytosolic glucocorticoid receptor (0.1 pmol). Bound receptor was determined by the immunoprecipitation test with RAM-Sepharose. The actual amount of antibody was determined previously by binding to RAM-Sepharose and measuring the protein eluted with 3.5 M NaSCN. The inset shows a Scatchard plot analysis of the data (B, bound antibody (determined from bound receptor); F, free antibody (total antibody minus mouse antibody). One receptor binding site was assumed for each antibody molecule.). Monoclonal antibodies secreted by clones 25 —■——■—; 49 —○——○; 57 —●——●—; 98 —▲——▲).

Table II. Influence of monoclonal antibodies on receptor binding to RAM-Sepharose and on binding of the receptor to DNA-cellulose

Monoclonal antibody	% Receptor bound to RAM-Sepharose[a]	% Receptor bound to DNA-cellulose[b]
25	49	60
49	75	59
57	55	59
98	42	63
None	6	63
None[c]	5	13[c]
25 + 57	53	n.d.
25 + 98	63	n.d.
57 + 98	65	n.d.
25 + 57 + 98	45	n.d.

[a]Binding measured by immunoprecipitation as described in Materials and methods.
[b]After incubation with antibody the receptor was heat-activated and binding to DNA-cellulose measured.
[c]Control which contained no antibody and was not heat-activated.

body. Therefore, one can conclude that the interaction of the receptor with the antibodies did not significantly reduce its ability to bind to DNA-cellulose.

Specificity of the antibodies

The glucocorticoid receptor of rat liver can occur in different forms depending on the conditions used for tissue homogenization. When the livers are frozen prior to homogenization, only the 40 000 mol. wt. form of the receptor, that we have purified to homogeneity, can be detected in the cytosol (Figure 4, and Westphal and Beato, 1980). For the preparation of the antibodies, however, the 90 000 mol. wt. form of the receptor was isolated from fresh liver cytosol

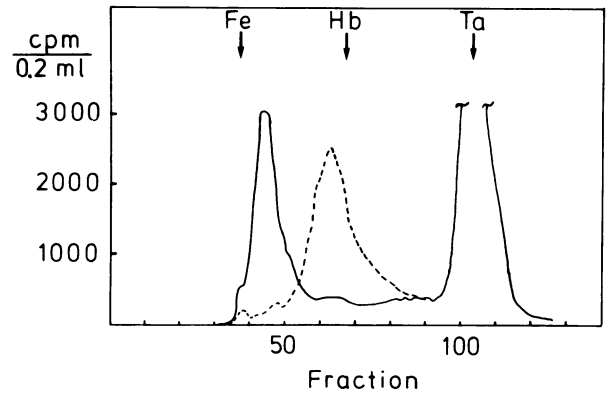

Fig. 4. Gel filtration of rat liver cytosol labelled with [^{3}H]triamcinolone acetonide. Cytosol was applied to a Sephdex G-150 column and eluted with buffer C as described in Materials and methods. ——— Cytosol from fresh liver. - - - - - - - Cytosol receptor from frozen liver. The arrows denote the elution volume of internal markers: Fe (Ferritin), Hb (hemoglobin), Ta (triamcinolone acetonide).

Table III. Specificity of four monoclonal antibodies against glucocorticoid receptor from various species and tissues

Species	Tissue	% Receptor binding[a]			
		Monoclonal antibody			
		25	49	57	98
Rat	liver fresh	100	100	100	100
	liver frozen	6	8	11	17
	lung	44	93	30	119
	brain	74	111	114	270
	thymus	27	53	34	40
Rabbit	liver	0.4	16	0.4	0.5
Mouse	liver	3.6	96	7.4	8.4
Guinea pig	liver	0.1	37	2.4	1.2
Hen	liver	1.7	96	0.9	2.6

[a]Binding was measured by the immunoprecipitation test as described in Materials and methods and is expressed as percentage binding of the antibodies to receptor from fresh rat liver.

(Figure 4; Wrange *et al.*, 1979). There is circumstantial evidence suggesting that the smaller form of the receptor originates from the larger one by proteolysis (Wrange and Gustafsson, 1978) and, therefore, it was of interest to test whether the antigenic determinants are present in both forms of the receptor. None of the monoclonal antibodies tested was able to bind significantly to the 40 000 mol. wt. form of the receptor (Table III), thus suggesting that the antigenic determinants are located in the region of the receptor that is lost during the generation of the 40 000 mol. wt. form from the 90 000 mol. wt. form.

Another interesting question was whether the glucocorticoid receptor in other tissues of the rat was recognized by the antibodies. To avoid possible proteolysis, homogenization of the fresh tissues was carried out in buffer containing 20 mM molybdate. As can be seen in Table III, the glucocorticoid receptors of rat brain and lung were bound by all four antibodies tested. Receptor from thymus was bound to a lesser extent.

The livers of other species (in Table III) contain glucocorticoid receptors which are recognized only by the IgG anti-

body. From the species tested, the mouse receptor appears to be immunologically closer to the rat than the other three species. This was unexpected because the monoclonal antibodies were generated in BALB/c mice, the same strain used as receptor source. It appears that these mice have produced antibodies reacting with their own glucocorticoid receptor. The liver receptors of all species were prepared in buffer containing molybdate, and gel filtration controls showed that they were not degraded into smaller forms. Thus, it seems that the IgM antibodies only recognize the receptor from rat liver, whereas the IgG antibody from clone 49 shows interspecies cross-reactivity.

Discussion

The experiments described here show the feasibility of preparing monoclonal antibodies against the rat liver glucocorticoid receptor. We have screened the antibodies by measuring binding of the receptor labelled with a radioactive steroid. This screening method does not allow the detection of antibodies directed against the steroid-binding site of the receptor. For this purpose, an alternative screening method should be used that detects the receptor protein itself, for instance, a spot-immunodetection test (Huet *et al.*, 1982).

Seven of the positive clones secreted IgM and one clone IgG antibodies. The affinity constants of four antibodies tested varied greatly within two orders of magnitude. The cross-reactivity studies show that clone 49 lgG antibody is very likely directed against an epitope distinct from that recognized by the IgM antibodies. Whether the IgM antibodies react with identical or different epitopes of the receptor molecule is still open. It could be demonstrated that all four antibodies tested recognize the glucocorticoid receptor from other rat tissues such as brain, lung, and thymus. None of the monoclonal antibodies tested was able to interact with the smaller form of the glucocorticoid receptor (mol. wt. 40 000), that contains the steroid-binding and DNA-binding domains (Westphal and Beato, 1980). Obviously, the antibodies described here are directed against antigenic determinants located in a third domain of the 90 000 mol. wt. receptor that is absent from the smaller form. Very recently, Carlstedt-Duke *et al.* (1982), have obtained similar results with rabbit polyclonal antibodies, and have defined an immunoactive domain of the receptor that can be separated from the other two domains by treatment with α-chymotrypsin. These authors could not raise antibodies against the other two domains in rabbits immunized with rat liver 90 000 mol. wt. glucocorticoid receptor. These findings agree with our results, but are in contrast to work by other groups, that have obtained rabbit antibodies able to recognize both forms of the glucocorticoid receptor (Govindan, 1979; Tsawdaroglou *et al.*, 1981). This discrepancy may be due to differences in the receptor preparations used for immunization, but taken together the available data suggest that the steroid and DNA binding domains are relatively conserved in rodents, whereas the third domain in the receptor molecule exhibits greater evolutionary variability.

With the limitation in mind, that our antibodies only recognize one domain of the receptor molecule, it seems that the glucocorticoid receptors of different tissues of the rat are immunologically very similar. This finding confirms previous observations with polyclonal antibodies to the glucocorticoid receptor (Okret *et al.*, 1981; Carlstedt-Duke *et al.*, 1982), and with monoclonal antibodies to the estradiol receptor (Greene *et al.*, 1980). Whenever negative results are obtained with this type of antibodies, it is necessary to demonstrate that the receptor is in its native 90 000 mol. wt. form, since the smaller form is not recognized. By this criterion, we found that at least the antigenic determinant that is recognized by the IgG of clone 49, is also well preserved in other species, whereas the antigenic determinants recognized by the other antibodies are not present in the glucocorticoid receptor from rabbit, mouse, guinea pig, and hen.

Since four of our monoclonal antibodies did not interfere with receptor binding to DNA, they could be used for the isolation of receptor-DNA complexes out of heterogeneous systems. This tool may allow us to investigate the interaction of the receptor with specific sequences on the DNA without the need for purification of the receptor protein.

Materials and methods

Receptor preparation

Receptor was purified from fresh rat livers as described by Wrange *et al.* (1979). The pyridoxal phosphate eluate from the second DNA-cellulose column was precipitated with 50% ammonium sulfate. The pellet (32 – 46 μg) was dissolved in 1 ml buffer B, and used for immunization.

Immunization

Two female BALB/c mice were immunized at 9 weeks of age with freshly prepared receptor. The first injection containing 23 μg of receptor material in Fruend's complete adjuvant was given s.c. at multiple sites on the back. Mice were challenged 3 weeks later by injection of 21 μg receptor material in Freund's incomplete adjuvant s.c. and i.p. After another 3 week-interval a final booster injection of 16 μg receptor material in phosphate buffer was administered i.v.

Cell fusion

Three days after the final immunization, mice were exsanguinized by cardiac puncture and splenectomized. Fusion of 1 x 10^8 spleen cells with 1 x 10^7 X63-Ag8.653 mouse myeloma cells (Kearney *et al.*, 1979) was achieved with 45% polyethylene glycol 4000 (Serva, Heidelberg) supplemented with 5% dimethyl sulfoxide (Merck, Darmstadt) according to the procedure of Galfrè *et al.* (1977). Fused cells at a concentration of 6 x 10^5/ml were cultured with 2.7 x 10^5 isogeneic feeder spleen cells in 24-well tissue culture cluster dishes (Costar No. 3524, Cambridge, MA). Each well contained the cell mixture in 1.5 ml (RPMI/1640 medium Seromed, München) with L-glutamine and sodium bicarbonate, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml amphotericin B, supplemented with 10% heat-inactivated fetal calf serum (Seromed). Medium was replaced by selective growth medium (0.1 mM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine in complete RPMI 1640 medium (Littlefield, 1964) over the next 3 days by siphoning off 1 ml of supernatant and adding 1 ml HAT medium. Within 2 – 3 weeks hybridomas were visible with the naked eye and cultures were then fed with HT medium (HAT without aminopterin). Hybridoma supernatants were screened for mouse immunoglobulin by ELISA. Specificity of monocloned antibodies for the glucocorticoid receptor was determined by an immunoprecipitation assay described below.

Cloning and expansion of hybridomas

Cultures of interest were cloned by limiting dilution using 96-well microtiter plates (Falcon No. 3040, Oxnard, CA) and rat thymocytes (3 x 10^6/ml) as feeder cells. At first, clones were expanded in tissue culture and aliquots were frozen in liquid nitrogen. Larger amounts of monoclonal antibodies were generated in ascites of BALB/c mice by inoculating hybridoma cells i.p. following treatment with pristane (Roth, Karlsruhe). Immunoglobulin obtained from ascitic fluid was partially purified by 2 – 3 sequential precipitations with 50% saturated ammonium sulfate and extensive dialysis against PBS.

Preparation of RAM-Sepharose 4B immunoadsorbent

Affinity chromatography-purified rabbit anti-moue IgG (RAM) antibodies were coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the procedure of Cuatrecasas (1970). To each ml of activated, packed, Sepharose 4B, 3.5 mg specific rabbit anti-mouse IgG was offered. The coupling efficiency was >98%. Prior to the first use, irreversibily binding antibodies of the adsorbent were saturated with irrelevant mouse immunoglobulin.

ELISA

Synthesis of mouse immunogobulin in hybridoma cultures was measured

by a double antibody sandwich ELISA. Polyvinylchloride microplates (Flow No. 77-173-05, McLean, VA) were coated with purified goat anti-mouse Ig. Bound mouse immunoglobulin from culture supernatants was detected using akaline phosphatase-labeled goat anti-mouse Ig. By employing class- and subclass-specific enzyme-conjugated anti-mouse antibodies, the immunoglobulin heavy chain isotype of selected clones was determined with the same assay (Hämmerling *et al.*, 1981).

Cytosol preparation

The livers of male Wistar rats, adrenalectomized 1 – 3 days before sacrifice, were perfused *in situ* through the portal vein with the homogenizing buffer. For preparation of the 90 000 mol. wt. receptor, the livers were at once minced, 1 – 1.5 volume buffer A (20 mM sodium phosphate, pH 7.0, 1 mM EDTA, 10% glycerol, 2 mM mercaptoethanol, and 50 mM NaCl) added and homogenized with a Teflon-glass homogenizer and centrifuged for 1 h with a minimum of 100 000 *g* at 2°C. The supernatant was incubated with 0.05 μM [^{3}H]triamcinolone acetonide, specific activity 26 or 37 Ci/mmol, at 0°C for 1 h. Unbound steroid was removed with 5% dextran-coated charcoal. The receptor concentration was 7 – 10 pmol receptor/ml cytosol. For preparation of the 40 000 mol. wt. receptor, the livers were minced, frozen in liquid nitrogen, and stored at – 80°C until use. The frozen liver was thawed in 1 – 1.5 volumes TSS buffer (0.25 M sucrose, 25 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 2 mM mercaptoethanol, 50 mM Tris-HCl, pH 7.5) and homogenized. The further procedure was exactly as described above.

For the cytosol preparation of other tissues of the rat and livers from other species, 1 volume molybdate buffer (10 mM Tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid pH 7.9 at 4°C, 1.5 mM EDTA, 0.25 M sucrose, 20 mM Na_2MoO_4) was added to the tissue before homogenization.

Immunoprecipitation tests

Screening. The screening of hybridomas secreting antibodies to the receptor was based on the detection of the streoid. 500 μl culture supernatant were added to 100 μl RAM-Sepharose (25% v/v) and rotated for 2 h at room temperature. The RAM-Sepharose was centrifuged off and washed three times with 1 ml PBS. Then 50 μl fresh liver cytosol, (labelled with [^{3}H]triamcinolone acetonide) and 400 μl PBS were added and rotated for a further 2 h at 40°C. After washing three times with 1 ml PBS, the pellet was transferred into a dioxane based scintillation fluid and the bound radioactivity measured.

Binding test. The titration of the antibodies produced in ascitic fluid was performed by adding increasing amounts of antibody to cytosol (0.1 pmol receptor) in a final volume of 30 μl. The actual concentration of the antibodies was determined previously by binding to the RAM-Sepharose followed by elution with 3.5 M NaSCN and protein determination (Schaffner and Weissmann, 1973). The mixtures of the receptor and antibodies were left at 4°C overnight. Then 50 μl RAM-Sepharose (25% v/v) was added and rotated for 2 – 3 h at 4°C. The immunoadsorbent was centrifuged off and washed three times with 100 μl 3.5 M NaSCN. The eluate was counted for radioactivity in a dioxane based scintillation fluid. The supernatants and washings were treated with 1 volume 0.5% dextran-coated charcoal, counted, and the value obtained was added to the NaSCN eluate to calculate the total amount of receptor (100% value). The control was a sample which contained buffer or normal mouse immunoglobulin instead of antibody.

Sedimentation studies

Cytosolic receptor (0.5 pmol) was incubated with 100 μl monoclonal antibody from ascitic fluid for 2 – 3 h and then layered on a 5 – 20% linear sucrose gradient in PBS containing 0.3 M NaCl. Two-drop fractions were collected and their radioactivity determined. Marker proteins were run in parallel gradients.

DNA-cellulose test

Cytosolic receptor (1 pmol) was incubated with saturating amounts of antibody (determined from Figure 4) overnight at 4°C. Then 4 volumes of buffer were added and the receptor activated for 30 min at 25°C. 600 μl of a 20% (v/v) DNA-cellulose (containing 67 μg DNA) was added and incubated for 15 min at 4°C. The DNA-cellulose was centrifuged off, washed twice with 1 ml buffer B, and then counted for radioactivity.

Gel filtration

Gel filtration was performed on a Sephadex G-150 column (2.4 x 69 cm) equilibrated in buffer C (20 mM Tris, 1 mM EDTA, 150 mM KCl, 10% glycerol, 2 mM mercaptoethanol, and 0.02% sodium azide, pH 7.4). 3 ml cytosol labelled with [^{3}H]triamcinolone acetonide was loaded on the column and eluted with buffer C at a flow rate of 13 ml/h. 3 ml-fractions were collected, aliquots of 0.2 ml taken off and measured for radioactivity.

Acknowledgements

We thank Mrs. Heidi Kaufmann-Schmidt for technical help during the receptor preparation. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 103, B1, C1, Be 759-4). H.M. Westphal receives a scholarship from the Deutsche Forschungsgemeinschaft.

References

Carlstedt-Duke,J., Okret,S., Wrange,Ö, and Gustafsson,J.-A. (1982) *Proc. Natl. Acad. Sci. USA,* **79**, 4260-4264.
Cuatrecasas,P. (1970) *J. Biol. Chem.*, **245**, 3059-3065.
Eisen,H.J. (1980) *Proc. Natl. Acad. Sci. USA,* **77**, 3893-3897.
Galfré,G., Howe,S.C., Milstein,C., Butcher,G.W., and Howard,J.C. (1977) *Nature,* **266**, 550-552.
Govindan,M.V. (1979) *J. Steroid Biochem.*, **11**, 323-332.
Greene,G.L., Fitch,F.W., and Jensen,E.V. (1980) *Proc. Natl. Acad. Sci. USA,* **77**, 157-161.
Gronemeyer,H., and Pongs,O. (1980) *Proc. Natl. Acad. Sci. USA,* **77**, 2108-2112.
Hämmerling,G.J., Hämmerling,U., and Kearney,J.F. eds., (1981) *Monoclonal Antibodies and T-Cell Hybridomas,* published by Elsevier, North-Holland, pp. 563-587.
Huet,J., Sentenac,A., and Fromageot,P. (1982) *J. Biol. Chem.*, **257**, 2613-2618.
Kearney,J.F., Radbruch,A., Liesegang,B., and Rajewsky,K. (1979) *J. Immunol.*, **123**, 1548-1550.
Köhler,G., and Milstein,C. (1975) *Nature,* **256**, 495-497.
Littlefield,J.W. (1964) *Science (Wash.),* **145**, 709-710.
Okret,S., Carlstedt-Duke,J., Wrange,Ö., Carlström,K., and Gustafsson, J.-A. (1981) *Biochim. Biophys. Acta,* **677**, 205-219.
Schaffner,W., and Weissmann,C. (1973) *Anal. Biochem.*, **56**, 502-514.
Tsawdaroglou,N.G., Govindan,M.V., Schmid,W., and Sekeris,C.E. (1981) *Eur. J. Biochem.*, **114**, 305-313.
Westphal,H.M., and Beato,M. (1980) *Eur. J. Biochem.*, **106**, 395-403.
Westphal,H.M., Fleischmann,G., and Beato,M. (1981) *Eur. J. Biochem.*, **119**, 101-106.
Wrange,Ö, Carlstedt-Duke,J., and Gustafsson,J.-A. (1979) *J. Biol. Chem.*, **254**, 9284-9290.
Wrange,Ö, and Gustafsson,J.-A. (1978) *J. Biol. Chem.*, **253**, 856-865.