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Monoclonal antibodies against the rat liver glucocorticoid receptor

(hybridoma/receptor structure/enzyme-linked immunosorbent assay/immunoprecipitation/immunoblotting)

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ABSTRACT **Splenic cells from one BALB/c mouse and one C57/BL mouse, immunized with purified rat liver glucocorticoid receptor (GR), were fused with the mouse myeloma cell line Sp 2/0-Ag 14. Screening for production of anti-GR-antibodies by the hybridomas was carried out with an enzyme-linked immunosorbent assay, using partially purified rat liver GR as antigen. Further screening was by a second-antibody immunoprecipitation assay using [^{3}H]triamcinolone acetonide–GR complex from rat liver cytosol as tracer. Hybridomas from 10 different microplate wells, positive in both assays, were successfully cloned by the limiting dilution method to monoclonality. The different origins of the monoclonal antibodies were confirmed by their various isoelectric points when analyzed by isoelectric focusing. Four of the monoclonal hybridoma cell lines secreted IgM antibodies; two, IgG1; three, IgG2a; and one, IgG2b. The GR–antibody complex was identified in glycerol density gradients by a shift of the 4S GR to an 8.5S or 19S GR–antibody complex when incubated with monoclonal IgG or IgM antibody, respectively. The 10 monoclonal antibodies recognized different determinants on the GR, all situated on that domain of the receptor that is separate from the ligand and DNA-binding domains. Also, the cross-reactivity to the mouse liver GR varied among the monoclonal antibodies. No cross-reactivity was observed to the human lymphocytic GR. NaDodSO$_4$ electrophoresis of a 0.5% pure GR preparation followed by immunoblotting using one of the monoclonal antibodies identified a single peptide with a molecular weight of 94,000, identical to the purified rat liver GR.**

Until recently, methods to study steroid receptor structure and mechanism of action have been dependent on the ability of the receptor protein to bind radiolabeled ligands. However, the glucocorticoid receptor (GR) has been reported to exist in an active and a nonactive form, respectively, in which only the former is capable of binding the ligand (1). Furthermore, steroid receptors are sensitive proteins that under improper experimental conditions may lose their steroid binding capacity (2). Using extensively purified steroid receptors, several groups have been able to raise antibodies against these proteins (3–17) and they have used the antibodies as tools to study receptor structure and function. Receptor antibodies make it possible to detect steroid receptors independently of their capacity to bind steroid. With regard to the GR, antibodies have been described by us (10, 12) and by others (13–17). We have used polyclonal antibodies, raised in rabbits, immunized with highly purified rat liver cytosolic GR preparations, to characterize the structure of the GR (10–12). With these antibodies, it was possible to characterize a non-ligand-binding domain of the GR that seems to be necessary for the biological function of the receptor (11). However, fine structure analysis of steroid receptor molecules is limited by the heterogeneity and low titer of the polyclonal antisera. In this paper, we describe the preparation and properties of 10 monoclonal mouse antibodies against the rat liver GR.

MATERIALS AND METHODS

Immunization. GR (40–60% pure) was prepared from rat liver cytosol as described by Wrange *et al.* (18), except that elution from the second DNA-cellulose column was carried out with 25 mM $MgCl_2$. The amount of GR injected was estimated from radioactivity assuming one [^{3}H]triamcinolone acetonide [TA, [9α-fluoro-11β,21-dihydroxy-16α,17α-(1-methylethylidenebis[oxy])pregna-1,4-diene-3,20-dione]-binding site per receptor molecule and a calculated molecular weight of the GR of 94,000 (19). GR was concentrated by trichloroacetic acid precipitation (20) and dissolved in 0.1–0.2 ml of phosphate-buffered saline (P_i/NaCl; 8 g of NaCl, 2.9 g of $Na_2HPO_4 \cdot 2H_2O$, 0.2 g of KH_2PO_4, and 0.2 g of KCl per liter, pH 7.4). For immunization, female BALB/c or C57/BL mice (Anticimex, Stockholm, Sweden), 8 weeks old, were used. The BALB/c mouse used for the fusion was immunized subcutaneously with 5 μg of GR emulsified in Freund's complete adjuvant (Difco). Booster injections were carried out 4 weeks later with 3 μg of GR emulsified in Freund's incomplete adjuvant (Difco) given subcutaneously and after another 2 weeks with 4 μg of GR given intraperitoneally. The mouse was left for 2 months before the last booster injections, consisting of 8 and 7 μg of GR, respectively, the latter including 25 μg of colchicin (Sigma), were given intravenously with a 1-day interval. The spleen was removed for fusion 4 days after the last booster injection. The C57/BL mouse was treated according to a similar immunization protocol, although 3–4 times higher doses of GR were given at each injection.

Cells and Media. The mouse myeloma cell line Sp 2/0-Ag 14 (21) was used as a fusion partner. This line was grown in Dulbecco's modified Eagle medium (DME medium) (Flow Laboratories). The medium was supplemented with 8% (vol/vol) heat-inactivated fetal calf serum (FCS) (GIBCO, Biocult), bensylpenicillin (400 international units/ml; Astra, Södertälje, Sweden), streptomycin (0.2 mg/ml; Novo, Copenhagen), 2 mM L-glutamine (Flow Laboratories), 1 mM sodium pyruvate (Flow Laboratories), bovine insulin (0.2 unit/ml; Sigma), oxaloacetic acid (0.15 mg/ml; Sigma), and 50 μM 2-mercaptoethanol (Sigma). DME medium was used after fusion with further addition of 0.1 mM hypoxanthine/0.2 μM aminopterin/1.6 mM thymidine (all from Sigma) (HAT medium). Feeder cells used were BALB/c macrophages, obtained by peritoneal lavage with 0.3 M sucrose.

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Abbreviations: GR, glucocorticoid receptor; TA, triamcinolone acetonide [9α-fluoro-11β,21-dihydroxy-16α,17α-(1-methylethylidenebis[oxy])pregna-1,4-diene-3,20-dione]; P_i/NaCl, phosphate-buffered saline.

Fusion and Cloning. Fusion was carried out by the method of Köhler and Milstein (20, 22), as modified by several authors (23–25). A spleen/myeloma Sp 2/0 cells ratio of 5:1 and 1 ml of 50% (wt/vol) polyethylene glycol 4000 (Merck's for gas chromatography) was used. After fusion, 3×10^5 fused cells and 5×10^3 macrophages were added to each microwell in a total volume of 0.2 ml of HAT medium.

Monoclonal antibody-producing hybridomas were obtained by cloning the cells twice at limiting dilution on a feeder layer of 5×10^3 macrophages per well. Evaluation of the monoclonality of the hybridomas was carried out according to principles for limiting dilution (26).

Large-scale production of monoclonal antibodies was carried out by growing hybridoma cells in culture or as ascites. Control ascites fluid was obtained in a similar way from Sp 2/0 cells.

Isoelectric Focusing. Electrofocusing was carried out as described in LKB Instruction Manual 1818A (LKB Produkter, Bromma, Sweden). After completed focusing, the pH gradient was measured by using a surface pH electrode and this was immediately followed by immunofixation for 60 min at room temperature with rabbit anti-mouse immunoglobulin (Dako Immunoglobulins, Copenhagen), previously adsorbed on fetal calf serum coupled to CNBr-activated Sepharose 4B (Pharmacia) and diluted 1:15 in P_i/NaCl. After washing with P_i/NaCl, (three 40-min periods), the gel was covered with several layers of Whatman 3MM chromatography paper and pressed for 20 min with a 1-kg weight. On the gel was layered a solution of protein A (Pharmacia) in P_i/NaCl for 45 min at room temperature. To the protein A was coupled horseradish peroxidase (Sigma) by the periodate method (27), and then the gel was washed and pressed with filter paper as described above and stained with 3,3'-diaminobenzidine (0.3 mg/ml; Sigma)/0.005% H_2O_2 in 0.04 M Tris·HCl (pH 7.6) for 20–30 min. The reaction was stopped by transferring the gel to water, and finally the gel was dried.

Test for Antibody Class. Class and subclass of the heavy chain of the monoclonal antibodies were determined by the Ouchterlony technique. Anti-mouse Ig class and subclass antisera—i.e., goat anti-IgM, -IgG1, -IgG2a, -IgG2b, and -IgA (Meloy Laboratories, Springfield, VA) and rabbit anti-IgG3 (Litton Bionetics)—were used.

Enzyme-Linked Immunosorbent Assay (ELISA). Anti-GR antibodies in serum or in hybridoma culture medium were assayed by a direct ELISA using purified rat liver cytosolic GR (30–80% purity) attached to the ELISA microtiter wells. Antibodies bound to the GR were detected by incubation with 50 μl of peroxidase-labeled rabbit anti-mouse immunoglobulin (Dako Immunoglobulins), diluted 1:50 in P_i/NaCl/0.05% Tween 20 (Merck)/0.5% bovine serum albumin (Sigma) for 30 min at 37°C. (For further details concerning the ELISA, see refs. 10–12.)

Preparation of Hormone–Receptor Complexes. [^{3}H]TA-labeled (specific activity, 31.3 or 37 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) cytosol was prepared in EPG buffer [20 mM sodium phosphate, pH 7.4/2 mM 2-mercaptoethanol/1 mM Na_2EDTA/10% (wt/vol) glycerol] as described (10). Limited proteolysis of the 6-nm rat liver GR to the 3.3-nm steroid- and DNA-binding GR fragment was carried out using α-chymotrypsin (28).

Second-Antibody Immunoprecipitation Assay. [^{3}H]TA–GR complex from rat liver cytosol (0.1 pmol) was incubated with 50 μl of hybridoma culture medium, serum, or ascites at various dilutions and 4 μl of normal mouse serum in a final volume of 0.2 ml of EPG buffer containing 0.15 M NaCl for 1.5–2 hr at 4°C. GR–antibody complex was precipitated by adding 50 μl of undiluted rabbit anti-mouse immunoglobulin (Dako Immunoglobulins).

After incubation for 30 min at 4°C, precipitates were pelleted by centrifugation and washed twice with 1 ml of EPG buffer containing 0.15 M NaCl and 100 μg of insulin/ml. Following dissolution of the pellets in 1 M NaOH and neutralization with 1 M HCl, radioactivity was measured. Nonspecific binding was determined in tubes containing [^{3}H]TA–GR complex and medium from Sp 2/0 cells or normal mouse serum. Samples were scored positive if precipitated radioactivity was 2–3 times the amount for nonspecific binding.

RESULTS

Three weeks after fusion, supernatants from the original microtiter wells were tested for anti-GR antibodies by a direct ELISA. Seven percent (34/480 wells) and 10% (79/769 wells) of the wells originating from the fusion with BALB/c and C57/BL mouse splenic lymphocytes, respectively, were positive by the ELISA. Of the 34 ELISA-positive supernatants from the BALB/c fusion, 3 (0.6% of total) supernatants were positive in a second-antibody immunoprecipitation assay using crude cytosolic [^{3}H]TA–GR complex as antigen. Cells from two of these three wells were successfully cloned at limiting dilution to monoclonality (monoclonal antibodies 1 and 2). Supernatant from 22 (2.9% of total) of 79 ELISA-positive wells originating from the fusion with C57/BL lymphocytes reacted with cytosolic [^{3}H]TA-labeled GR. Eight of these showed stable and high anti-GR-immunoactivity. Hybridomas from these eight wells were cloned (monoclonal antibodies 3–10) as described above.

Characterization of the monoclonal antibodies by isoelectric focusing in agarose gels (Fig. 1) showed different isoelectric points for each of the 10 monoclonal antibodies. This shows that all 10 antibodies are different and that they originate from different stem cells.

Characterization of the monoclonal antibodies using class- and subclass-specific anti-mouse immunoglobulins showed that four of the monoclonal antibodies were IgM, two were IgG1, three were IgG2a, and one was IgG2b (Table 1).

The titers (defined as the dilution of unpurified ascites that immunoprecipitated 50% of 0.1 pmol of rat liver cytosolic [^{3}H]TA–GR complex in the second-antibody immunoprecipitation assay) of the monoclonal antibodies derived from ascites varied from 1:40 to 1:700 (Table 1 and Fig. 2). IgG antibodies occurred at higher titers compared with IgM antibodies (Table 1).

Cross-reactivity and specificity of the monoclonal antibodies were tested by glycerol density gradient centrifugation in 0.15 M salt or by immunoprecipitation assay or both. The 4S cytosolic [^{3}H]TA–GR complex shifted to a sedimentation coefficient of 8.5 S when incubated with any of monoclonal IgG antibodies 5–9 (Fig. 3). This suggests that each antibody binds to only one site per receptor molecule. Addi-

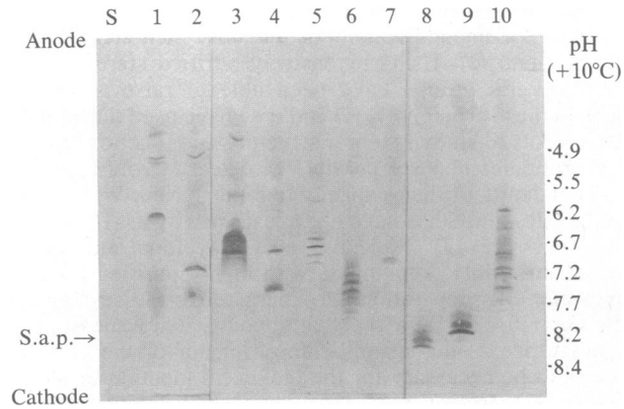

FIG. 1. Isoelectric focusing in agarose gels of culture supernatants containing monoclonal antibodies 1–10, respectively. Culture medium from Sp 2/0 cells (lane S) served as control. S.a.p., sample application point.

Table 1. Summary of properties and cross-reactivities of the monoclonal antibodies

Monoclonal antibody	Antibody		Cross-reactivity against mouse liver GR‡	Binding to protein A§
	Class*	Titer†		
1	IgM	~1:100	−	−
2	IgM	~1:40	−	−
3	IgM	~1:150	++	−
4	IgM	~1:40	−	−
5	IgG1	~1:150	+	−
6	IgG1	NT	NT	NT
7	IgG2a	~1:400	+	++
8	IgG2a	~1:700	−	+
9	IgG2a	~1:600	−	+
10	IgG2b	~1:300	++	−

NT, not tested.
*Determined in an Ouchterlony assay.
†Defined as the dilution of unpurified ascites that immunoprecipitates 50% of 0.1 pmol of rat liver cytosolic [^{3}H]TA–GR complex in a second-antibody immunoprecipitation assay.
‡Tested with the second-antibody immunoprecipitation assay against the GR with a Stokes radius of 6 nm.
§Tested by an ELISA using protein A conjugated to horseradish peroxidase as a marker.

tion of a second IgG antibody shifted the sedimentation peak to about 10.5 S (Fig. 3), while addition of a monoclonal IgM antibody shifted the 8.5S peak to a 20–21S peak (data not shown). Any combination (except 7 and 9) of two of the monoclonal IgG antibodies (monoclonal antibodies 5–9) gave a 10.5S peak. When monoclonal antibodies 7 and 9 were mixed, no additional displacement of the 8.5S [^{3}H]TA–GR–antibody complex was seen. This is probably explained by steric hindrance between antibodies recognizing closely located antigenic determinants. The possibility that the two monoclonal antibodies recognize the same antigenic determinant seems to be ruled out by the different cross-reactivities to mouse GR (Table 1).

In contrast to the 8.5S peak obtained when [^{3}H]TA–GR

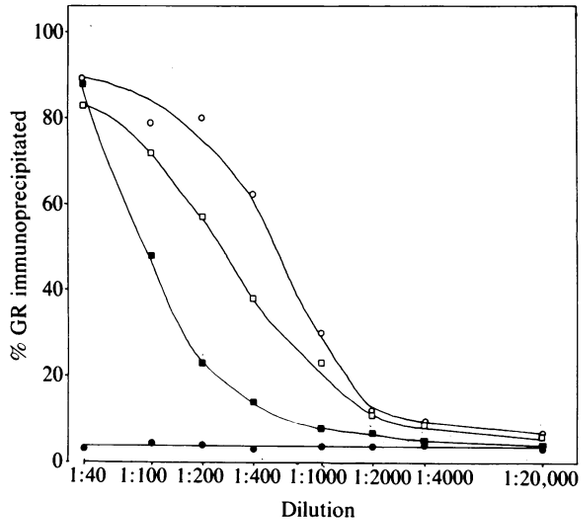

FIG. 2. Titration curves for ascites fluids containing monoclonal antibodies assayed by immunoprecipitation. [^{3}H]TA–GR complex (0.1 pmol) from crude rat liver cytosol was incubated with various dilutions of unpurified ascites fluids containing monoclonal antibody 1 (■), 8 (○), or 10 (□). ●, GR was precipitated in the presence of ascites from a mouse injected with Sp 2/0 cells. Each point represents the mean of duplicate assays. The dilution given on the abscissa is the final dilution of ascites fluid in the incubation mixture. The amount of GR immunoprecipitated is calculated as percentage of total GR present in the incubation mixture.

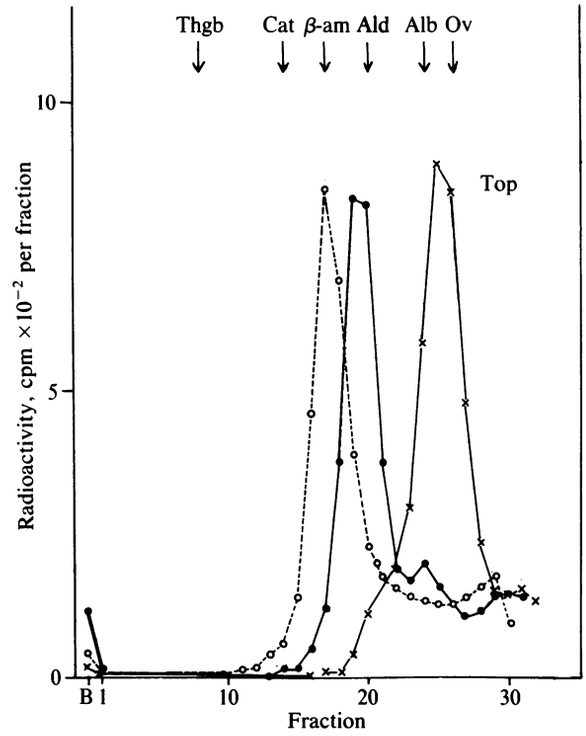

FIG. 3. Sedimentation profile of [^{3}H]TA–GR complex from crude rat liver cytosol treated with monoclonal IgG antibody. [^{3}H]TA–GR complex (0.1 pmol) from crude rat liver cytosol was incubated with 50 μl of unpurified Sp 2/0 ascites (×), 25 μl of unpurified ascites containing monoclonal antibody 8 (●), or 25 μl of unpurified ascites containing monoclonal antibody 8 together with 25 μl of unpurified ascites containing monoclonal antibody 9 (○). The incubation mixture was layered on 5-ml 12–50% (wt/vol) glycerol density gradients containing EPG buffer, 0.15 M NaCl, and 100 μg insulin/ml and centrifuged for 17 hr at 200,000 × *g* at 0–2°C. Gradients were emptied from the bottom of the tube in 180-μl fractions. "B" indicates the radioactivity that adhered to the bottoms of the tubes. Sedimentation coefficients for the ^{14}C-labeled standards are Ov, ovalbumin (3.5 S, ref. 29); Alb, bovine serum albumin (4.5 S, ref. 29); Ald, aldolase (7.9 S, ref. 29); β-am, β-amylase (9.4 S, ref. 30); Cat, catalase (11.3 S, ref. 29); Thgb, thyroglobulin (19.2 S, ref. 31).

complex was incubated with monoclonal IgG antibodies 5–9, incubation with monoclonal IgG antibody 10 gave a single or double peak between 8.5 and 11 S, varying among several experiments (data not shown). This suggests that a receptor–antibody complex consisting of more than one receptor molecule per antibody, or vice versa, is formed (see *Discussion*).

Although all IgG antibodies recognized different epitopes, no additional peak displacement (i.e., of the 10.5S peak; cf. above) was seen on the gradients when three monoclonal IgG antibodies were incubated with the [^{3}H]TA–GR complex. This is probably explained by steric hindrance.

The 4S cytosolic [^{3}H]TA–GR complex shifted to a 19S [^{3}H]TA–GR–antibody complex when incubated with one of monoclonal IgM antibodies 1–4 (Fig. 4), and combination of two monoclonal IgM antibodies gave an additional displacement of the sedimentation peak to about 24 S (Fig. 4). This suggests that the monoclonal IgM antibodies recognize different antigenic determinants on the receptor molecule.

The 6-nm rat liver GR can be resolved into three domains—a steroid-binding domain, a DNA-binding domain, and a domain that also seems to be necessary for the biological function of the GR (11, 32). Seven of seven of our polyclonal rabbit antisera tested bound to the latter domain but not to the other two GR domains (12). The same domain specificity characterizes all 10 monoclonal antibodies described in this report; none of these monoclonal antibodies

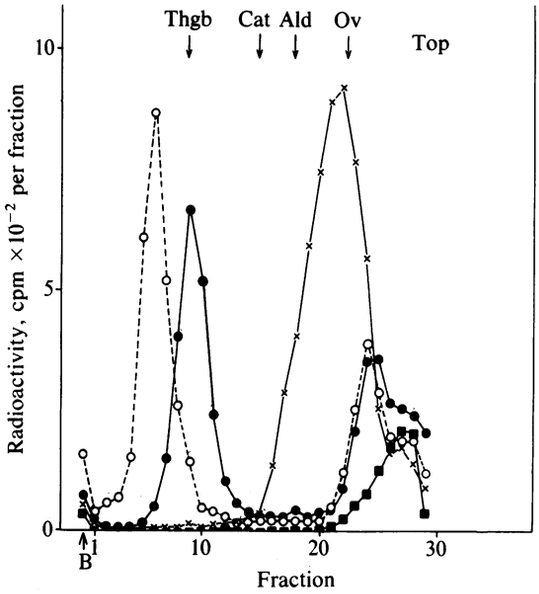

FIG. 4. Sedimentation profile of [^{3}H]TA–GR complex from crude rat liver cytosol treated with monoclonal IgG antibody. [^{3}H]TA–GR complex (0.1 pmol) from crude rat liver cytosol was incubated with 250 μl of control ascites, Sp 2/0 (×), 30 μl of ascites containing monoclonal antibody 1 (●), or 30 μl of ascites containing monoclonal antibody 1 plus 170 μl of ascites containing monoclonal antibody 2 added to the incubation mixture 1 hr later (○) for a total incubation time of 2 hr at 4°C. A parallel incubation of cytosol with [^{3}H]TA in the presence of a 200-fold excess of unlabeled TA was also analyzed (■). The incubation mixtures were layered on 12–50% (wt/vol) glycerol density gradients and centrifuged as described in Fig. 3.

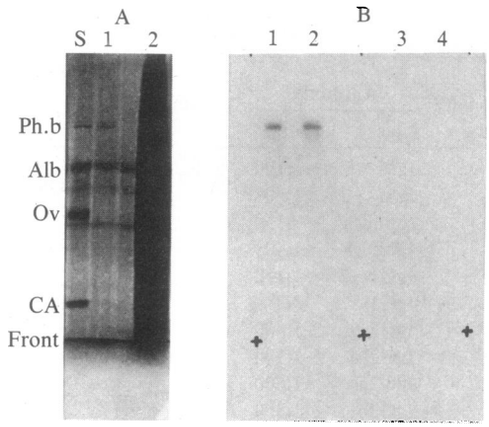

FIG. 5. Immunoblotting of rat liver cytosolic GR separated by NaDodSO$_4$/PAGE. Rat liver GR (0.25 μg per well) from a 50% pure GR preparation (*A*, lane 1, and *B*, lanes 1 and 3) or from a parallel GR preparation to which 25 μg of cytosolic protein was added (final purity of GR, 0.5%; *A*, lane 2, and *B*, lanes 2 and 4) were trichloroacetic acid precipitated and analyzed by 10% (wt/vol, polyacrylamide) NaDodSO$_4$/PAGE as described (20). (*A*) Electrophoretic pattern after silver staining of the gel. Electrophoretic transfer to nitrocellulose sheets (type GS, pore size 0.22 μm; Millipore) was carried out according to Burnette (33). The sheets were then coated with 5% (wt/vol) bovine serum albumin (Sigma) in P$_i$/NaCl for 1 hr at room temperature, and this was followed by incubation with culture supernatant containing monoclonal antibody 7 (*B*, lanes 1 and 2) overnight at room temperature. Incubation with culture supernatant from Sp 2/0 cells served as control (*B*, lanes 3 and 4). After the overnight incubation, the cells were washed with P$_i$/NaCl/0.05% Nonidet P-40 for four 30-min periods, incubated with ^{125}I-labeled protein A (300,000 cpm/ml, New England Nuclear, specific activity, 10 μCi/μg) for 1 hr at room temperature, and again washed with P$_i$/NaCl/0.05% Nonidet P-40/1 M NaCl for four 30-min periods. After drying, the nitrocellulose sheets were exposed to LKB Ultrofilm in an X-Omat film casette with a regular intensifying screen (Kodak) for 48 hr at −70°C. Molecular weights of standard proteins (lane S) (Pharmacia) were as follows; phosphorylase b (Ph. b), 97,400 (34); bovine serum albumin (Alb), 67,000; ovalbumin (Ov), 45,000 (35); carbonic anhydrase (CA), 30,000.

bind to the 3.3-nm steroid- and DNA-binding entity (data not shown). None of the monoclonal antibodies showed any cross-reactivity to the human lymphocytic GR. However, 4 of 9 monoclonal antibodies tested showed cross-reactivity to the mouse liver GR (Table 1). No difference in antibody reactivity to rat liver as compared with rat lung GR was seen for either monoclonal antibodies 1 or 2. Furthermore, monoclonal antibody 1 did not cross-react with the estrogen or progestin receptor from rat uterus (data not shown). The monoclonal antibodies of IgG2a class bound *Staphylococcus aureus* protein A (Table 1).

The immunoblotting technique (33) was used to test the monospecificity of the monoclonal antibodies. A 50% pure GR preparation (Fig. 5*A*, lane 1) and a parallel sample to which 100 times the amount of cytosolic protein was added (final degree of receptor purity, 0.5%; Fig. 5*A*, lane 2) were separated by NaDodSO$_4$/PAGE (36). Parallel preparations were analyzed by immunoblotting using monoclonal antibody 7 (Fig. 5*B*, lanes 1 and 2). A single immunogenic peptide with a molecular weight of 94,000, identical to the purified GR, was found, regardless of the degree of purity [Fig. 5*B*, lane 1 (50% pure GR) and lane 2 (0.5% pure GR)]. No immunoactivity was observed when identical GR preparations were incubated with medium from Sp 2/0 cells (Fig. 5*B*, lanes 3 and 4). This experiment showed that monoclonal antibody 7 is highly monospecific.

DISCUSSION

In this report, we describe the preparation and characterization of 10 monoclonal anti-rat liver GR antibodies originating from two separate fusions. Eight monoclonal antibody-producing hybridoma cell lines were obtained from the C57/BL mouse, whereas two cell lines were derived from the BALB/c mouse, which was injected with one-quarter to one-third as much GR as the former animal. The two cell lines from the BALB/c mouse produced IgM antibodies, while 6 of the 8 antibodies secreted from cell lines originating from the C57/BL mouse were of the IgG class. The lower titers observed for monoclonal IgM antibodies when compared with IgG antibodies (Table 1) is in accordance with the lower affinity to antigen usually displayed by IgM when compared with IgG antibodies (37).

When analyzed by isoelectric focusing, all monoclonal antibodies except no. 10 showed fewer than three or four bands on the gel (Fig. 1). Monoclonal antibody 10 also behaved differently from the other monoclonal antibodies by forming antigen–antibody complexes with various sedimentation coefficients when analyzed by density gradient centrifugation. This might raise some doubt with regard to the monoclonality of antibody 10. After thorough recloning of the antibody 10-producing cell line, however, all subclones secreted antibodies of the same subclass (IgG2b) and with the same isoelectric focusing pattern, indicating that antibody 10 is indeed monoclonal. The sedimentation profiles might be explained by monoclonal antibody 10 recognizing a repetitively expressed determinant on the GR or by one monoclonal antibody 10 binding to two GR molecules. A similar phenomenon was observed by Moncharmont *et al.* (7). One of their monoclonal antibodies against the estrogen receptor formed a 2:1 complex of receptor to antibody in the presence of excess receptor.

All different antigenic determinants that are recognized by the 10 monoclonal antibodies are situated on that domain of the receptor that is separate from the steroid- and DNA-

binding entity. The same specificity is shown by polyclonal anti-GR antibodies raised by us (10–12) and by Eisen and coworkers (38). Also, the monoclonal antibodies against the rat liver GR, recently described by Westphal *et al.* (17), showed the same specificity. However, Govindan and Sekeris (13) have raised polyclonal antibodies that also recognize the steroid- and DNA-binding GR entity (cf. above). This discrepancy may perhaps be explained by different purification or immunization techniques.

Some of the monoclonal antibodies described in this paper cross-reacted with the mouse liver GR but none bound to the human lymphocytic GR, suggesting that some antigenic determinants are highly species specific. Westphal *et al.* (17) found that only one out of eight monoclonal anti-rat GR antibodies recognized GR from other species. Similar observations were made by Greene *et al.* (5), who raised monoclonal antibodies against the estrogen receptor from calf uterus. They found no cross-reactivity to estrogen receptor from several other species tested. However, their monoclonal antibodies to the human estrogen receptor cross-reacted with estrogen receptor from several other mammalian species (6). In contrast to this, some monoclonal antibodies raised against the calf uterus estrogen receptor by Moncharmont *et al.* (7) cross-reacted with the human receptor.

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