Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 7719–7723, December 1982
Biochemistry

Development of hybridomas secreting monoclonal antibodies to the chicken intestinal 1α,25-dihydroxyvitamin D_3 receptor

(steroid receptor/vitamin D_3 hormone/sedimentation/human intestinal 1α,25-dihydroxyvitamin D_3 receptor/breast cancer cell 1α,25-dihydroxyvitamin D_3 receptor)

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Communicated by C. S. Marvel, September 27, 1982

ABSTRACT **Four hybridomas that secrete monoclonal antibodies to the chicken intestinal cytoplasmic 1α,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] receptor have been obtained. Splenic lymphocytes, derived from two male Lewis rats expressing serum antireceptor activity after repeated immunization with a partially purified preparation of this protein, were fused with two nonsecreting murine myeloma cell lines, SP2/0-Ag14 and P3-X63-Ag8.653. Viable hybrids were screened for anti-chicken intestinal 1,25-$(OH)_2D_3$ receptor activity by incubation of hybrid media with receptor–hormone complex; this was followed by immunoprecipitation with rabbit anti-rat IgG. Of 1,724 hybridomas assayed by this technique, 4 were positive (2 derived from each animal) for the secretion of an antireceptor immunoglobulin molecule. After cloning by limiting dilution, the cell lines (designated SP2/0-4A5, P3-8C8, SP2/0-8D3, and SP2/0-9A7) were expanded into suspension culture. Antibody-induced alterations in the sedimentation pattern of the native 1,25-$(OH)_2D_3$ receptor, coupled with Ouchterlony double-diffusion techniques, indicate that SP2/0-4A5 secretes an IgG2a, SP2/0-9A7 produces an IgG2b, and P3-8C8 secretes an IgG. In contrast, SP2/0-8D3 was found to synthesize an IgM. The monoclonal antibodies react with both occupied and unoccupied chicken intestinal receptor and nuclear receptors, and they crossreact with 1,25-$(OH)_2D_3$ receptors from a wide variety of tissue and cultured cell types, including human 1,25-$(OH)_2D_3$ receptors. These immunological reagents should prove valuable in the elucidation of the molecular action of 1,25-$(OH)_2D_3$.**

The mechanism of action of the secosteroid 1α,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] is currently considered to be similar, if not identical, to that of other typical genomic-acting steroid hormones (1, 2). Consistent with this hypothesis is the fact that all known target tissues of vitamin D_3, including intestine (3), bone (4), and kidney (5), contain specific, high-affinity cytosolic receptor proteins that interact with chromatin (6) and are thought to bind to DNA (7). This association apparently alters specific transcriptional events in favor of mineral-regulating proteins such as vitamin D_3-dependent calcium-binding protein (8).

A prerequisite to the elucidation of vitamin D action is a more complete understanding of the biochemical and physicochemical nature of the 1,25-$(OH)_2D_3$ receptor molecule. Because of the lack of cellular abundance and inherent chemical instability of the receptor, its purification from chicken intestine has proven to be difficult (7, 9). We have described elsewhere (10) the use of a partially purified chicken intestinal receptor preparation, containing a predominant protein of 64,000 daltons, as immunogen in eliciting serum antireceptor response in two different male Lewis rats. We report here the successful polyethylene glycol-mediated fusion of mouse myeloma cells with splenic lymphoid cells from these two rats to obtain stable hybridomas that secrete monoclonal antibodies to the chicken intestinal receptor.

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MATERIALS AND METHODS

Reagents. 1α,25-Dihydroxy[23,24(n)-^{3}H]vitamin D_3 (120 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was generated from 25-hydroxy[23,24(n)-^{3}H]vitamin D_3 (Amersham) as described (11).

Animals. Rhode Island Red cockerels were raised on a vitamin D_3-deficient diet (12) supplemented with 3% $CaCO_3$ for 3–4 weeks prior to decapitation and intestine removal. Inbred male Lewis rats (Microbiological Associates, Bethesda, MD) were used for immunization and as a source of control serum, splenic lymphocytes, kidney, and duodenal mucosa.

Cultured Cell Lines. Mouse SP2/0-Ag14 and P3-X63-Ag8.653 myeloma cells were maintained in logarithmic growth phase between 1 and 10×10^5 cells per ml in a humidified atmosphere of 95% air/5% CO_2. Cells were grown in Iscove's modified Dulbecco's medium (IMD medium, GIBCO) supplemented with 15% fetal calf serum (Sterile Systems, Logan, UT). Rat osteogenic sarcoma (ROS 17/2.8, a gift of G. Rodan), embryonic human intestine (IN-407, ATCC), and other cell lines were grown by using standard cell culture techniques and harvested with trypsin/EDTA at confluency.

Buffers. The following buffers were used: STKMET, 0.25 M sucrose/50 mM Tris·HCl, pH 7.4/25 mM KCl/5 mM $MgCl_2$/1 mM EDTA/12 mM thioglycerol; KETT, 10 mM Tris·HCl, pH 7.4/1 mM EDTA/12 mM thioglycerol and variable molar concentrations of KCl as indicated (e.g., KETT-0.1 M); phosphate-buffered saline, 20 mM sodium phosphate, pH 7.2/0.15 M NaCl; HPB, 10 mM Tris·HCl, pH 7.5/2 mM EDTA/0.5 mM EGTA/0.15 mM spermine/0.5 mM spermidine/5 mM dithiothreitol.

Purification of 1,25-$(OH)_2D_3$ Receptor and Immunization of Lewis Rats. Partial purification of the 1,25-$(OH)_2D_3$ receptor from 10 kg of rachitic chicken intestines is described in detail elsewhere (10); partial purification yielded 412 μg of 13% pure receptor. The immunization procedures and schedule for the two Lewis rats, designated F and C, also are detailed elsewhere (10). Significant serum titers of antireceptor antibody were detectable 7–10 days after the third injection. Four months after initiation of immunization and 3 days prior to spleen removal,

Abbreviations: 1,25-$(OH)_2D_3$: 1α,25-dihydroxyvitamin D_3; IMD medium, Iscove's modified Dulbecco's medium; HAT, hypoxanthine/aminopterin/thymidine; KETT, 10 mM Tris·HCl, pH 7.4/1 mM EDTA/12 mM thioglycerol (KETT-0.1 = KETT with 0.1 M KCl; KETT-0.15 = KETT with 0.15 M KCl, etc.); HPB, 10 mM Tris·HCl, pH 7.5/2 mM EDTA/0.5 mM EGTA/0.15 mM spermine/0.5 mM spermidine/5 mM dithiothreitol.

the animals each received a final intravenous injection (28 μg, rat F; 54 μg, rat C) in 1 ml of phosphate-buffered saline.

Cell Fusion. Spleens were removed aseptically from donor rats and placed into sterile Petri dishes containing IMD medium. Single cell suspensions were obtained by gentle Dounce homogenization and the cells were collected by centrifugation (400 × *g* for 10 min). Mouse myeloma cells were harvested in midlogarithmic phase (6–8 × 10^5 cells per ml) and washed in IMD medium.

Fusion of spleen cells with both myeloma cell lines SP2/0-Ag14 and P3-X63-Ag8.653 was performed by using the techniques of Kohler and Milstein (13) as modified by McKearn *et al.* (14): rat F, three sequential fusions (9 × 10^7 splenic lymphocytes and 25 × 10^6 myeloma cells per fusion); rat C, four sequential fusions (9 × 10^7 splenic cells and 2 × 10^7 myeloma cells per fusion). Fusion was accomplished by brief centrifugation of the spleen and myeloma cells (6 min) at 25°C in 30% polyethylene glycol 1,000 (Sigma); this was followed by slow dilution in IMD medium. Cells were centrifuged, resuspended in Iscove's selective hypoxanthine/aminopterin/thymidine (HAT) medium [Iscove's supplemented with 10% NCTC 109 (GIBCO)/20% heat-inactivated fetal calf serum/bovine insulin at 8 mg/liter/oxaloacetic acid at 132 mg/liter/0.1 mM hypoxanthine/0.4 μM aminopterin/pyruvate at 50 mg/liter/30 μM thymidine] containing penicillin and streptomycin, and equally dispersed into 96-well microtiter plates (Costar, Cambridge, MA) as follows: rat F (17 plates) and rat C (22 plates). The cells then were incubated at 37°C in a humidified atmosphere of 95% air/5% CO_2. Selective HAT medium (≈50 μl) was added to each well on days 2, 4, and 7 after fusion. Wells that contained hybridoma growth (10–21 days after fusion) then were assayed for anti-1,25-$(OH)_2D_3$ receptor activity (described below) as the media became visibly acidic. Wells that tested positive for antireceptor activity were cloned by limiting dilution on a Lewis rat splenic lymphocyte feeder layer (10^6 cells per well). All positive clones from each line were expanded and then either were frozen (5–10 × 10^6 cells per ml in 90% fetal calf serum/10% dimethyl sulfoxide and stored in liquid N_2) or expanded further into spinner culture. The hybridomas that had been frozen also were recovered as antireceptor antibody-secreting cells when thawed.

Preparation of 1,25-$(OH)_2D_3$ Receptor Complex. Chicken intestinal 1,25-$(OH)_2D_3$ receptor was partially purified by Polymin P fractionation from intestinal homogenates (7). Receptor was dissolved in KETT-0.15 and incubated overnight with 10 nM 1,25-$(OH)_2[^3H]D_3$ (120 Ci/mmol) at 2°C to form receptor–hormone complex. Just prior to use in the screening immunoprecipitation assay and in sedimentation studies, the labeled complexes were subjected to gel filtration on Sephadex G-25 (Pharmacia) to remove free hormone. Rat intestinal and kidney 1,25-$(OH)_2D_3$ receptors were prepared as described (10).

Preparation of Estrogen and Glucocorticoid Receptors. Estrogen (MCF-7) and glucocorticoid (rat liver) receptors were prepared as described (10).

Double-Antibody Immunoprecipitation Assay. Hybridoma media, as well as immunized rat serum, were assayed for the presence of antireceptor antibody by incubation with 1,25-$(OH)_2[^3H]D_3$ receptor complex; this was followed by immunoprecipitation basically as described by Greene *et al.* (15). Receptor complex (0.05–0.2 pmol) first was incubated in KETT-0.15 with antiserum (1–10 μl) or hybridoma medium (2–100 μl; 100 μl during screening) for 4–6 hr at 2°C in a total volume of 0.4 ml containing 5 μl of normal rat carrier serum. After the initial incubation, enough rabbit anti-rat IgG (whole molecule, Sigma) was added to each tube (100 μl) to bind all rat Ig and was allowed to react overnight. The precipitate was collected by centrifugation, washed, and dissolved in 0.1 M NaOH (50 μl) prior to estimation of radioactivity in 5 ml of ACS scintillant (Amersham) on a Beckman 7500 liquid scintillation spectrometer.

Sedimentation Analysis. 1,25-$(OH)_2[^3H]D_3$ receptor complexes (0.02–0.2 pmol) from either Polymin P-fractionated chicken intestine, rat tissue cytosols, or cultured cell nuclear extracts were incubated with immune serum (1–10 μl) or hybridoma medium (2–200 μl) for 4 hr at 2°C in a final volume of 0.42 ml in KETT-0.15. After incubation, the solutions were layered onto 4.8-ml gradients of 10–30% sucrose prepared in KETT-0.3 and centrifuged at 2°C in an SW 50.1 rotor (Beckman) at 265,000 × *g* for 18 hr prior to fractionation. Radioactivity associated with the gradient pellet was solubilized in 0.1 M NaOH before the addition of scintillant to the lower section (0.5 cm) of the tube. The external sedimentation standard used to determine the sedimentation coefficient was 6.6S rabbit IgG (Sigma).

Ouchterlony Double-Diffusion Analysis. Media from the expanded hybridomas (100–1,000 ml) were precipitated twice with phosphate-buffered ammonium sulfate (40% of saturation), concentrated, and reacted against commercial preparations (Pel Freez) of rabbit or goat anti-rat IgG subclasses or anti-rat μ chains. Overnight reaction was generally sufficient to determine classification, with rabbit anti-rat IgG (whole molecule) as control.

RESULTS

Lewis Rat Antisera. Antibodies to the 1,25-$(OH)_2D_3$ receptor were raised in two male Lewis rats by immunization with a partially purified preparation of chicken intestinal receptor (10). As illustrated in Fig. 1, reaction of the serum antibody with Polymin P-fractionated chicken receptor displaced the radioactivity from the normal 3.3S position of the native receptor to much heavier sedimenting units (≈13 S). The induction of antisera to the 1,25-$(OH)_2D_3$ receptor permitted us to attempt the generation of hybridomas secreting monoclonal antibodies.

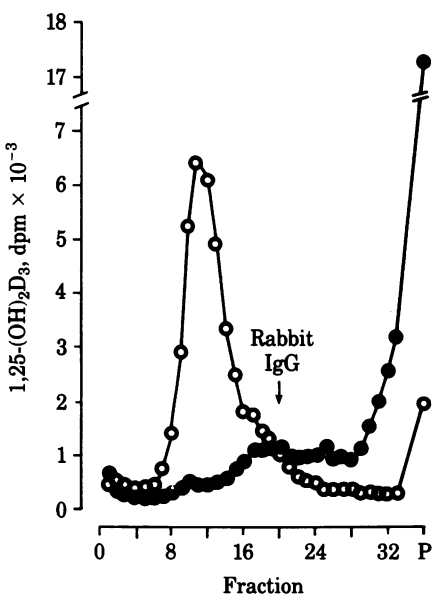

FIG. 1. Detection of anti-1,25-$(OH)_2D_3$ receptor antibodies in immunized Lewis rat serum by sedimentation analysis. Chicken intestinal 1,25-$(OH)_2D_3$ receptor (0.1 pmol) was incubated with 10 μl of Lewis rat preimmune serum (○) or 10 μl of Lewis rat immune serum (●) from rat F for 4 hr at 2°C and then was analyzed on 10–30% sucrose gradients prepared in KETT-0.3. Identical data were obtained with antiserum from rat C. P is the pellet at the tube bottom.

Table 1. Mouse–rat hybridomas producing monoclonal antibody to 1,25-$(OH)_2D_3$ receptor

Lewis rat, spleen	Myeloma, cell line	Hybridomas: Wells, no. Assayed	Wells, no. Positive	Clones, no. positive	Clone designation	Rat Ig subclass
F	SP2/0-Ag14	441	1	20/21	SP2/0-4A5	IgG2a
F	P3-X63-Ag8.653	569	1	*	P3-8C8	IgG
C	SP2/0-Ag14	569	2	15/16	SP2/0-8D3	IgM
				21/37	SP2/0-9A7	IgG2b
C	P3-X63-Ag8.653	145	0	—	—	—

* Not yet cloned.

Creation of Spleen Cell–Mouse Myeloma Hybrids Positive for Antireceptor Activity. The results of fusion experiments, utilizing spleen cells derived from both animals, are summarized in Table 1. Of 1,010 (rat F) and 714 (rat C) hybrids that arose after fusion and were screened by immunoprecipitation assay, 4 (0.24%) were positive for the presence of antireceptor antibody. Three of these wells were cloned by limiting dilution on a spleen cell feeder layer, whereupon reassay of the hybridomas revealed a high percentage of single cells positive for antireceptor monoclonal antibody. The cell lines were designated SP2/0-4A5, SP2/0-8D3, and SP2/0-9A7 after the parent wells, with the uncloned line designated as P3-8C8, and were shown by Ouchterlony double-diffusion analysis to secrete specific immunoglobulin molecules, as indicated in Table 1. Interestingly, although three of the monoclonal antibodies are of the IgG class, one (from SP2/0-8D3) is an IgM.

Characteristics of the Interaction Between Chicken Intestinal Receptors and Monoclonal Antibodies. The reactivity of chicken intestinal receptor and specific antibodies was first characterized by sedimentation analysis on 10–30% sucrose gradients by using media derived from all four cell lines. As depicted in Fig. 2A, media derived from SP2/0-4A5 caused a shift in sedimentation of receptor from the 3.3S region of the gradient to the 7–8S region, indicative of a single receptor–immunoglobulin complex. Reactivity of the SP2/0-4A5 media with unoccupied receptor was indistinguishable from that with occupied receptor (data not shown), when determined by sedimentation analysis that was followed by labeling of the gradient fractions with 1,25-$(OH)_2[^3H]D_3$. Media derived from both the P3-8C8 and the SP2/0-9A7 cell lines produced identical profiles to those of Fig. 2A when similarly reacted with occupied receptor (data not shown). However, as depicted in Fig. 2B, the interaction of 1,25-$(OH)_2D_3$ receptor with SP2/0-8D3 media clearly resulted in a complex that sedimented to the bottom of the gradient, confirming Ouchterlony analysis that indicated the presence of an IgM molecule (Table 1). These results verify that the newly created cell lines are producing monoclonal antibodies against chicken intestinal 1,25-$(OH)_2D_3$ receptor.

Reactivity of Human Embryonic Intestinal and Rat Osteogenic Sarcoma Receptor with Serum and Monoclonal Antibody. Receptors for 1,25-$(OH)_2D_3$ have been identified in a host of cell lines (16). Thus, it was of interest to determine the reactivity, and thus usefulness, of both serum and monoclonal antibodies with other 1,25-$(OH)_2D_3$ receptors. As illustrated in Fig. 3, both the serum and 4A5 antibodies quantitatively displace receptor derived from cultured human embryonic intestine (Fig. 3A) and rat osteogenic sarcoma (Fig. 3B). Furthermore, because the receptor in these studies was recovered from the nucleus after whole cell incubation with 1,25-$(OH)_2[^3H]D_3$, the antibodies clearly bind to the nuclear form of this protein.

Crossreactivity and Specificity of the Monoclonal Antibodies. Crossreactivities similar to those pictured in Fig. 3 were observed with 1,25-$(OH)_2D_3$ receptors from a variety of other tissue and culture cell sources, as summarized in Table 2.

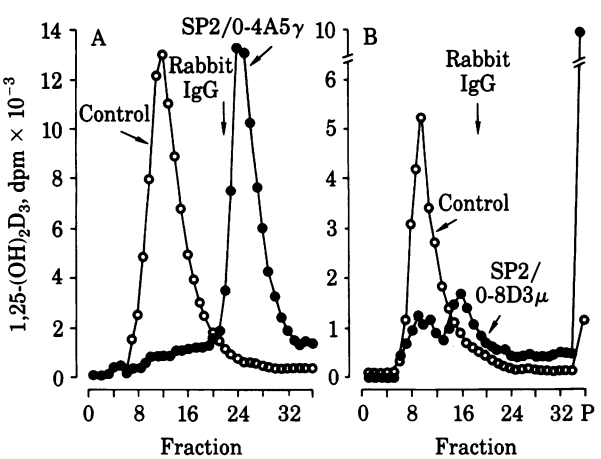

FIG. 2. Interaction of SP2/0-4A5 and SP2/0-8D3 monoclonal antibodies with chicken intestinal 1,25-$(OH)_2D_3$ receptor. (A) 1,25-$(OH)_2D_3$ receptor complex (0.2 pmol) was incubated with either 50 μl of control medium (○) or 5 μl of SP2/0-4A5 clonal medium (●) for 4 hr at 2°C and then was analyzed on 10–30% sucrose gradients in KETT-0.3. (B) Receptor (0.1 pmol) was incubated with either 100 μl of control medium (○) or 150 μl derived from SP2/0-8D3 clonal medium (●) and was analyzed as above. P as in Fig. 1.

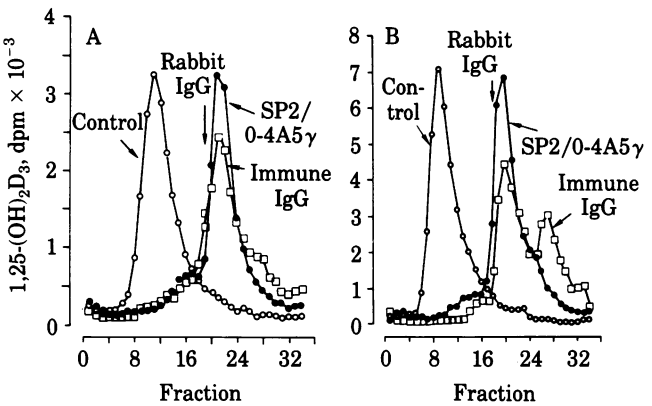

FIG. 3. Crossreactivity of Lewis rat antiserum and SP2/0-4A5 monoclonal antibody with nuclear 1,25-$(OH)_2D_3$ receptors from human embryonic intestine (IN-407) and rat osteogenic sarcoma (ROS 17/2.8). Nuclear 1,25-$(OH)_2D_3$ receptors were obtained from the cultured cell lines by incubation of 10^8 cells (10^7 cells per ml) with 2 nM 1,25-$(OH)_2[^3H]D_3$ suspended in cell culture media containing 1% fetal calf serum/0.1% ethanol for 1 hr at 37°C. Labeled cells were washed and homogenized in HPB. The nuclei were isolated by centrifugation and then were extracted in KETT-0.3 to obtain the nuclear 1,25-$(OH)_2D_3$ receptor fraction. Receptor complex was reacted with either 10 μl of preimmune Lewis rat serum (○), 2 μl of immunized Lewis rat serum (□), or 20 μl of SP2/0-4A5 clonal medium (●) for 4 hr at 2°C and then was sedimented on 10–30% sucrose gradients in KETT-0.3. (A) IN-407 receptor (0.05 pmol); (B) ROS 17/2.8 receptor (0.1 pmol).

Table 2. Specificity and crossreactivity of monoclonal antibodies to chicken 1,25-$(OH)_2D_3$ receptors

Receptor	Monoclonal antibody			
	4A5	9A7	8C8	8D3
1,25-$(OH)_2D_3$ receptors				
Intestinal tissue				
Chicken	+	+	+	+
Rat	+	+	ND	−
Kidney tissue				
Rat	+	+	ND	−
Cultured cells				
Rat pituitary (GH_3)	+	ND	ND	ND
Rat bone (ROS 17/2.8)	+	+	−	−
Pig kidney (LLC-PK_1)	+	+	+	−
Human breast (MCF-7)	+	+	+	−
Human intestine (IN-407)	+	+	+	−
Glucocorticoid receptor				
Liver				
Rat	−	−	ND	−
Estrogen receptor				
Cultured cell				
Breast cancer (MCF-7)	−	−	ND	−

* ND, not determined.

Clearly, the 4A5 and 9A7 antibodies react with all of the 1,25-$(OH)_2D_3$ receptors tested. In contrast, binding of the 8C8 monoclonal antibody to receptors is variable, and the 8D3 antibody is selective only for chicken receptor. Importantly, the antibodies are specific; they do not react with either the glucocorticoid or estrogen receptors.

DISCUSSION

The technique of lymphoid–myeloma fusion derived by Kohler and Milstein has seen wide application since first devised in 1975 (13). Recently, Greene *et al.* (15, 17) obtained a series of rat–mouse hybridomas that secrete antibodies to estrophilin, which constitutes an initial utilization of this technique in the steroid hormone receptor field. In this communication, we summarize our own work in developing monoclonal antibodies to the chicken intestinal 1,25-$(OH)_2D_3$ receptor, a protein that is presently considered to be similar both physically and functionally to other steroid hormone receptors.

The experiments reported here demonstrate the feasibility of using partially purified 1,25-$(OH)_2D_3$ receptor from chicken intestine to elicit a measurable immune response in Lewis rats (Fig. 1). Furthermore, they illustrate the subsequent application of hybridoma technology to obtain clonal cell lines that secrete monoclonal antibodies to the chicken intestinal receptor for 1,25-$(OH)_2D_3$ (Table 1). An immunoassay (15), which utilizes radiolabeled 1,25-$(OH)_2D_3$ as a marker for receptor and double-antibody precipitation as a means of quantitating antibody-bound receptor complex, provides a simple method whereby the media from hybrids arising through fusion could be screened for the presence of specific antireceptor immunoglobulins. This technique led to the detection of four positive wells, although the nature of the screening assay precluded the detection of antibody that caused hormone displacement. After cloning, three of the four newly created cell lines (SP2/0-4A5, SP2/0-8D3, and SP2/0-9A7) were expanded into suspension culture from which large quantities of monoclonal antibodies have been derived. Further, each appears to be highly stable and has been consistent in antibody secretion for over 10 months. Moreover, in recent experiments, two of the hybrids (SP2/0-4A5 and SP2/0-9A7) have been successfully introduced into athymic mice as antibody-secreting ascites tumors.

1,25-$(OH)_2[^3H]D_3$ not only serves as a radioactive label for detecting antibody–receptor complexes with the double-immunoprecipitation assay but it allows for an examination of this interaction via sedimentation analysis. This usefulness implies that receptor–antibody formation does not lead to rapid hormone dissociation. The sedimentation characteristics of serum antibody complexed with chicken intestinal receptor (Fig. 1) suggest that the antiserum is polyclonal and probably contains several immunoglobulins that bind to receptor. This serum antibody profile is similar to that seen for other steroid receptors (18, 19). In contrast, reactivity of receptor with either 4A5, 9A7, or 8C8 antibodies, without exception, leads to migration in the 7–8S region (Figs. 2 and 3). This indicates a complex composed of a single molecule of receptor with a molecule of immunoglobulin (13, 17) and demonstrates the monovalent nature of these antibodies. Reactivity of 8D3 antibody reveals the presence of an IgM (Fig. 2), although the multivalent nature of this molecule has yet to be determined. These classifications are also confirmed through direct Ouchterlony double-diffusion analysis with antisera directed against rat μ chains and specific subclasses of rat IgG (Table 1).

Several of the monoclonal antibodies described here also bind to a number of tissue and cell line-derived 1,25-$(OH)_2D_3$ receptors (Fig. 3 and Table 2). Furthermore, because the cell line receptors were obtained by nuclear extraction, it can be concluded that the antibodies also bind to the nuclear form, although this is not a prerequisite to binding. Finally, reactivity of the 4A5 monoclonal antibody with the unoccupied chicken receptor (data not shown) was identical to that for occupied receptor (Fig. 2*A*). These observations are especially significant, because they indicate that the monoclonal antibodies that we have obtained will be generally applicable in the biochemical investigation of 1,25-$(OH)_2D_3$ receptors regardless of cell or species origin or the presence of bound hormone. However, because both 8C8 and 8D3 antibodies display limited reactivity to 1,25-$(OH)_2D_3$ receptors from sources other than chicken, some degree of microheterogeneity among these receptors is apparent.

There are a number of important applications of these antibodies to experimentation involving the physiology and biochemistry of the 1,25-$(OH)_2D_3$ receptor. Immunocytochemical techniques should readily provide insights into (*i*) the subcellular location of the 1,25-$(OH)_2D_3$ receptor, (*ii*) the distribution of receptor among various organs and cell types, and (*iii*) potential receptor correlates with biological effects in target cells. The development of hormonal ligand-independent assays should permit investigation into the physiology and pathology of receptors. For example, it should be possible to study the hormonal influence on receptor population, characterize the nuclear processing of receptor, and identify potential molecular defects in the 1,25-$(OH)_2D_3$ receptor in specific clinical states such as vitamin D-dependent rickets type II (20). Finally, the use of immunoaffinity chromatography may provide the means for homogeneous isolation of the 1,25-$(OH)_2D_3$ receptor (21), thereby allowing a detailed study of the physical and biochemical properties necessary for ultimate elucidation of this receptor's function.

The authors gratefully acknowledge the helpful contributions of Suzanne Chandler, Dr. Ruthanne Kibler, Dr. Darrell Anderson, and, in particular, Dr. David O. Lucas, whose suggestions were invaluable during the course of this work. This research was supported by National Institutes of Health Grants AM 15781 to (M.R.H.) and AM 06249 to (J.W.P.).

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