MONOCLONAL ANTIBODIES TO THE β-SUBUNIT OF HUMAN CHORIONIC GONADOTROPHIN

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Human chorionic gonadotrophin (HCG) is the specific hormone of pregnancy [1]. HCG is a glycoprotein with mol.wt. of 58-60 kD, consisting of α and β-subunits with mol. wt. of 18 and 30 kD respectively.

Immonologic tests are available to determine the early stage of pregnancy and for the diagnosis of trophoblastic tumors (chorionepithelioma, hydatidiform mole, etc.), based on the discovery of HCG in the body fluids (blood and urine) [2]. Until recently antibodies for these test systems were obtained from the blood sera of animals immunized with HCG. In recent years monoclonal antibodies (MAB) to β-HCG, obtained by the hybridoma technique [7], have been used for these purposes in the West, in conjunction with radioimmunoassay and enzyme immunoassay. By the use of MAB to β-HCG the reagents can be standardized and a test system not cross-reacting with HCG can be developed.

The aim of this investigation was to obtain hybridomas producing MAB to β-HCG and to characterize these antibodies, which are essential for the development of test systems for use in the USSR for HCG immunoassay in the body fluids as immunologic tests for the early diagnosis of pregnancy and of trophoblastic tumors.

EXPERIMENTAL METHOD

To obtain hybridomas, BALB/c mice were immunized with the official pharmacopoeal preparation of HCG (from Moscow Endocrine Factory). The course of immunization consisted of three injections of the preparation (1500 U) in Freund's complete adjuvant, at intervals of 4 weeks. Before fusion of the cells the mice were reimmunized by a single injection of HCG in phosphate buffer solution, pH 7.2 (2000 U). The hybridomas were obtained by fusion of spleen cells of immunized mice with cells of myeloma line X-63.Ag8.653 by the method described in [4] in our own modification. The supernatants of the growing hybrid clones were tested for the presence of MAB to β-HCG by indirect enzyme immunoassay (EIA). For this purpose flat-bottomed 96-well plates (Moscow Factory) were sensitized overnight at 4°C with 30 U of HCG in 1 ml or with 0.1 μg of β-HCG in 1 ml of 0.05 M carbonate-bicarbonate buffer, pH 9.6. EIA was carried out by the method described in [12] in a reaction volume of 60 μl per well.

To obtain MAB in preparative amounts, hybridoma cells were grown in the form of ascites in the peritoneal cavity of BALB/c mice. MAB were isolated from the ascites fluid by salting out with ammonium sulfate followed by ion-exchange chromatography on DEAE-52 (Whatman, England) in a gradient of (0.005-0.25 M) sodium-phosphate buffer, pH 8.0 [4]. The concentration of MAB purified in this way was determined by measuring optical density at 280 nm (E = 14.3 for mouse IgG_1) [5]. The subclass of MAB obtained was established by Ouchterlony's double immunodiffusion test, using antiserum against subclasses of heavy chains of mouse immunoglobulins (Serotec, England). Electrophoresis of purified MAB also was carried out under reducing and nonreducing conditions in polyacrylamide gel (PAG) in the presence of sodium dodecylsulfate [10], together with isoelectric focusing in a thin layer of PAG on prepared plates (LKB, Sweden) with range of pH 5.5-8.5, by the technique recommended by the firm. The specificity of the

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TABLE 1. Characteristics of MAB

MAB	Myeloma	Antigen	Isotype of MAB	PI	Content of MAB in 1 ml ascites fluid, mg	Specificity
D_1	X—63.Ag8,653	HCG	Ig61	5,8	3,6	β-HCG
D_2	The same	The same	»	6,5	7,3	The same
D_3	» »	» »	»	6,5	3,5	» »
D_4	» »	» »	»	6,8	5,2	» »
D_5	» »	» »	»	6,7	6,3	» »

TABLE 2. Reaction of MAB with Human and Animal Gonadotrophic Hormones

Clone	β-HCG	Gonadotrophic hormone									
		HCG	HLH	HFSH	HTTH	BLH	BFSH	PLH	PFSH	ECG	ELH
D_1	+	+	—	—	—	—	—	—	—	—	—
D_2	+	+	—	—	—	—	—	—	—	—	—
D_3	+	+	—	—	—	—	—	—	—	—	—
D_4	+	+	—	—	—	—	—	—	—	—	—
D_5	+	+	—	—	—	—	—	—	—	—	—

Legend. +) Positive, −) negative reaction.

MAB thus obtained was tested by indirect EIA with human luteinizing hormone (HLH), human thyrotrophic hormone (HTTH), human follicle-stimulating hormone (HFSH), hog and cow luteinizing hormone (LH) (IWF-LHP2-0011, B4-2018, East Germany), hog FSH (Burns and Sigma, USA), cow FSH (NIH-ESH-B1, Bethesda, USA), horse CG (IWF-PMSG-18-G-129, East Germany), and horse LH (Sigma, USA). To develop a system for RIA for determination of HCG, β-HCG was labeled with ^{125}I by the method of Hunter and Greenwood [6]. To determine HCG in blood serum, an RIA system based on MAB was used, whereas to determine HCG in urine, a solid-phase RIA system was used. To establish the mutual arrangement of the epitopes recognized by the MAB under investigation, MAB produced by clone D_2 were conjugated with horseradish peroxidase by the method in [11]. The mutual arrangement of the epitopes was established by EIA on HCG: Unlabeled MAB produced by clones D_1-D_5 in a concentration of 100 μg/ml was incubated initially with HCG, and after washing, a conjugate of MAB with peroxidase (1.5 μg/ml) was added to the wells and EIA carried out.

EXPERIMENTAL RESULTS

As a result of hybridization of spleen cells of a hyperimmune mouse (titer of antibodies to HCG in EIA 10 days after the third injection of antigen was 1:25,600) with myeloma cell line X-63.Ag8.653, five hybrid clones producing MAB to β-HCG were obtained. After cloning by the limiting dilutions method hybridoma cells of monoclones D_1, D_2, D_3, D_4, and D_5 were injected into mice in order to obtain preparative amounts of MAB. The titer to HCG in the ascites fluid was 1:204,800. After purification of the MAB from ascites fluid the quantity of MAB produced from 1 ml of ascites fluid was determined by the method described above (Table 1). All MAB belonged to the IgG_1 isotype and on PAG electrophoresis under nonreducing conditions they gave one band corresponding to protein with mol. wt. of 160 kD, whereas under reducing conditions they gave two bands, one of which corresponded to protein with mol. wt. of 50 kD (heavy chain), the other to one of 25 kD (light chain). On isoelectric focusing each specimen of MAB gave one set of bands, proving that they were monoclonal. It was also discovered (Fig. 1) that clones D_2 and D_3 give the same set of bands. Consequently, D_2 and D_3 are subclones of the same clone.

The specificity of MAB was tested by indirect EIA, using human and animal gonadotrophic hormones as antigens. The results are given in Table 2.

The results in Table 2 are evidence that the MAB we obtained react specifically with β-HCG. An RIA test system for determination of HCG in the blood serum and urine of patients with chorionepithelioma or with other trophoblastic tumors and also for the diagnosis of early pregnancy in women was developed on the basis of MAB produced by clone D_2. β-HCG-^{125}I was used as the labeled antigen. A competitive test system with the use of secondary antibodies has been developed for analysis of β-HCG in blood serum and used in clinical practice at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. A solid-phase competitive RIA system in which the monoclonal antibodies were fixed to polystyrene test tubes was developed for analysis of HCG in urine. The sensitivity of both systems was 5 mIU/ml and the coefficients of variation in the series of samples about 5%. More than 300 determinations have been made with the aid of these test systems and have shown close correlation with the course of the disease (chorionepithelioma) and also with TBG (trophoblastic β-1 globulin), another marker of trophoblastic tumors.

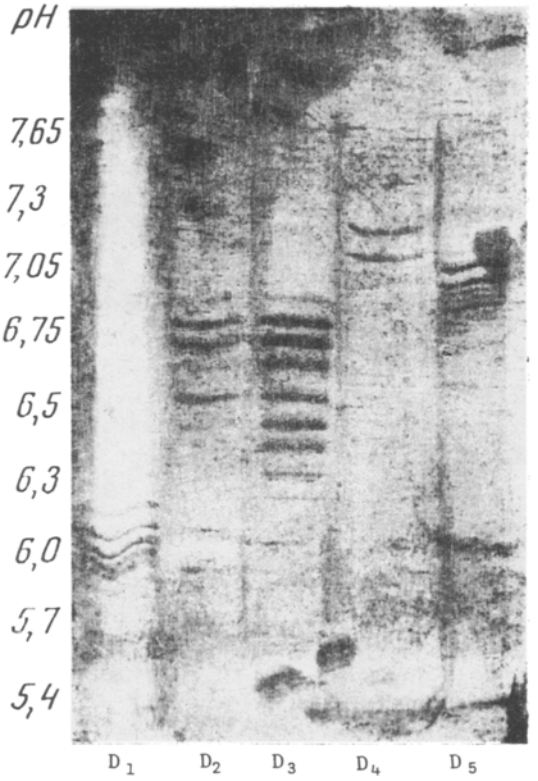

Fig. 1. Isoelectric focusing of monoclonal antibodies. D_1-D_5) Monoclonal antibodies of corresponding clones.

Four hybridomas producing MAB to β-HCG, belonging to the IgG_1 isotype, were thus obtained. RIA test systems for determination of HCG in blood and urine have been developed on the basis of MAB produced by clone D_2 for the early diagnosis of pregnancy and of certain malignant diseases and which can be used widely in medical practice. It has been shown by competitive EIA that antibodies produced by clones D_1, D_3, D_4, and D_5 inhibit binding of a conjugate of D_2 with antigen. Consequently, all these MAB are aimed against the same antigenic region [3]. It must be pointed out that we came across an interesting case of the response of a mouse to the official HCG preparation. As a result of hybridization of the spleen cells of this mouse with the myeloma cell line we obtained clones producing MAB against one specific determinant for β-HCG. Already 11 antigenic determinants on the HCG molecule have been described in the literature and the majority of clones whose description has been published produce MAB that cross react with HLH [8, 9].

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