Preparation of Monoclonal Antibodies against Human Thyrotropin Suitable for Diagnostic Systems and Their Use in Clinical Practice

L. K. Koryazova, G. I. Kovalevskaya, E. R. Sigal, T. A. Pozdnyakova, E. N. Petrova, V. E. Samoilova, and R. G. Vasilov

UDC 615.357:577.175.3:616.006-007:616.153.96-078.33

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 10, pp. 433-435, October, 1994
Original article submitted August 19, 1994

Original methods are presented for immunizing mice with preparations of human thyrotropin and for testing primary clones of producer hybridomas. Twelve monoclonal antibodies specific to six different regions of the antigen are obtained. A test system for enzyme immunoassay is developed on the basis of two antibodies, providing for detection of hypo- and hyperthyreoidism in clinical practice.

Key Words: *monoclonal antibodies; test system; thyrotropin*

Diseases of the thyroid are now the most widespread endocrine pathology after diabetes mellitus. In all the developed countries measurement of human thyrotropin (thyroid-stimulating hormone, TSH) on the basis of immunometric test systems is used as a primary or screening test in the assessment of thyroid status.

The study was aimed at obtaining monoclonal antibodies (MAB) specific to spatially separated determinants of TSH and at the development, on their basis, of a clinical method of enzyme immunoassay (EIA) for hormone determination in human serum.

MATERIALS AND METHODS

Female BALB/c mice aged from 6 weeks to 12 months were used in the study. A prolonged scheme of immunization with thyrotropin was developed [1]; we chose the subcutaneous method of injection and the following doses as being optimal: 30 μg for injection together with Freund's adjuvant and 20 μg for latex-immobilized antigen.

At the first stage immunization was performed with a highly purified TSH preparation obtained at the Laboratory of Preparative Chromatography and Ultrafiltration of the Institute of Biotechnology [5]. The last two immunizations were performed with preparations from Sigma and from the Research Endocrinological Center of the Russian Academy of Medical Sciences alone or in the form of a complex with T2-8 MAB. The complex was obtained by incubating T2-8 MAB with TSH in an equimolar ratio (weight/weight 3:1) in 1 ml physiological saline at 4°C for 14 h or at 37°C for 1 h. Four days before hybridization the final immunization was performed intravenously by injecting 25 μg of antigen in physiological saline.

The clones obtained were tested using the TSH preparation obtained at the Institute of Biotechnology and at the Research Endocrinological Center and with that purchased from Sigma.

EIA [1] and indirect precipitation were used as the optimal tests for anti-TSH MAB. Sepharose

Laboratory of Molecular Immunology, Institute of Biotechnology, Moscow. (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

0007-4888/94/0010-1133$12.50

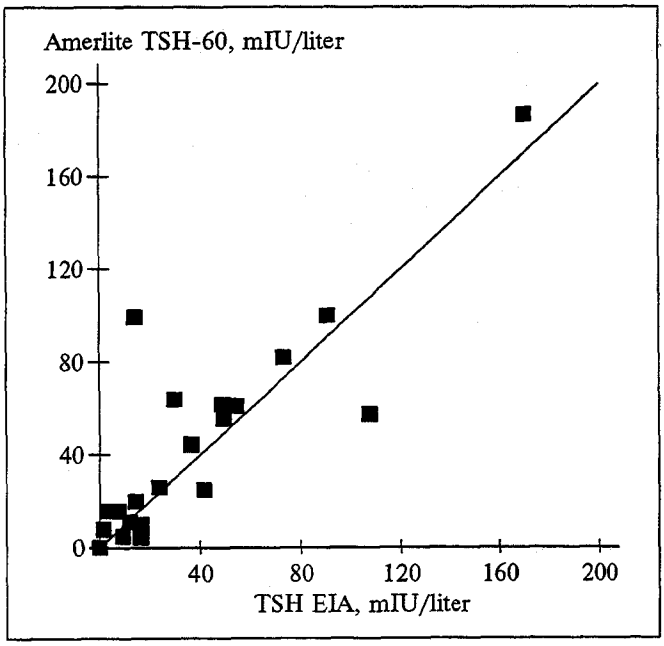

Fig. 1. Correlation between two-center TSH EIA and immunoluminescent Amerlite TSH-60 system. 352 samples of human serum were studied; the function is $y=0.945x+0.481$.

4B saturated with polyclonal rabbit antibodies to murine immunoglobulins was used in immunoprecipitation. Incubation was performed in one stage (simultaneous addition of supernatant labeled with ^{125}I-TSH and of the affinity sorbent) at 37°C during 1 h.

The epitope specificity of the antibodies produced by the primary hybridoma clones which were obtained after immunization with the TSH-T2-8 complex was compared with that of the earlier obtained T2-8 MAB using two approaches: studying the competition between MAB in the culture supernatant and horseradish peroxidase-labeled T2-8 and assessing the additive effect for simultaneous application of the supernatant and purified T2-8 to polystyrene plates with immobilized thyrotropin. In the first method T2-8 were used in a concentration of 150-200 ng/ml, resulting in a half-maximum binding of TSH. In the second case T2-8 were used in an excess concentration, which under the given experimental conditions constituted more than 2 μg/ml.

RESULTS

Pronounced heterogeneity is a specific feature of thyrotropin, as well as of other representatives of the glycoprotein hormones [6]. The use of different TSH preparations for immunization and testing ensured that all high-affinity anti-TSH antibodies secreted by the selected stable clones were specific to the antigenic determinants contained in all the test preparations of thyrotropin, i.e., they were typical of the majority of natural TSH isoforms.

When a prolonged scheme of immunization was used, the anti-TSH titer of the serum of immune mice attained the maximum (1.3×10^6) after 4 months of immunization. Immobilization of the antigen on a solid-phase substrate (latex) made it possible to reduce the dose from 30 to 20 μg and the frequency of injection of the immunogen from once every two weeks to once a month, as well as to dispense with adjuvants.

The use of two-center EIA and of indirect radioimmunoprecipitation enabled us to obtain a set

TABLE 1. Major Characteristics of MAB against Human TSH

MAB	Isotype	K_{aff}, $\times10^{-9}$ M^{-1}	Interaction between MAB and related hormones and β-subunit of TSH, %						
			LH	FSH	HCG	β-TSH	swine TSH	bovine TSH	specificity
T2-2	IgG1	1.0	0.08	0.06	0.06	1.8	47.7	39.8	αβ 1
T2-9	IgG1	2.2	0.07	0.06	0.06	5.7	63.1	50.7	αβ 1
T3-5	IgG1	2.5	0.07	0.08	0.06	7.9	87.9	70.5	αβ 1
T4-5	IgG1	1.5	0.05	0.06	0.04	4.5	55.2	30.7	αβ 1
T4-6	IgG1	1.7	0.08	0.07	0.06	6.0	64.5	46.2	αβ 1
T2-8	IgG1	12.0	0.06	0.06	0.05	5.8	40.2	31.9	αβ 1
T2-3	IgG2b	6.6	0.31	0.28	0.30	2.5	18.7	5.9	αβ 1-1
T3-3	IgG1	5.0	5.70	6.80	4.50	20.1	88.7	72.0	αβ 1-2
T5	IgG1	6.6	0.11	0.09	0.08	19.7	10.0	3.7	β
T6	IgG1	5.2	101.6	102.5	113.8	1.1	34.3	31.8	α
T8-2	IgG2b	8.2	0.08	0.06	0.05	1.2	6.8	4.3	αβ 2
T8-3	IgG1	7.6	0.07	0.06	0.05	7.5	23.1	20.7	β

Note. K_{aff}: affinity coefficient; LH: luteinizing hormone; FSH: follicle-stimulating hormone; HCG: human chorionic gonadotropin.

of antibodies recognizing the antigen in solution as early as at the stage of primary clones. In the first stage of the study, as a result of a series of hybridization, we obtained MAB to overlapping or closely situated antigenic determinants [1]. Analysis of the epitope specificity in competitive EIA demonstrated that a highly immunogenic region is conformational in nature and belongs to the dimer formed by the subunits, i.e., to the native TSH molecule rather than to the β-subunit, as was suggested by the findings of Benkirane *et al.* [4]. Evidently, this contradiction is due to specificities of the thyrotropin preparations and of the genotype of animals used for immunization.

The development of two-center EIA requires MAB specific to spatially separated antigenic determinants. Masking of the immunodeterminant region on the surface of TSH during immunization with T2-8 increased the likelihood of formation of MAB against other, less immunogenic epitopes on the surface of the hormone molecule. The use of competitive and additive EIA with T2-8 enabled us to detect clones producing such antibodies before the stage of recloning. The number of such MAB was some 70% of the total number of antibodies obtained.

As a result, we obtained a set of MAB specific to 6 antigenic regions on the surface of the TSH molecule [1]. Their main characteristics are presented in Table 1. The majority of antibodies react with the αβ 1 conformational immunodominant region. The regions αβ 1, 1-1, and 1-2 partially overlap. Two antigenic determinants are situated on the hormone subunits, both free and in the dimeric form (α,β). The majority of MAB react with swine and bovine TSH preparations, which corroborates published data on their structural and immunological homology [6].

We found that the T2-8 and T8-3 pair of MAB provides for the maximum sensitivity and specificity of analysis and is optimal for developing EIA for TSH determination [2]. We used it to design a test system whose analytical parameters meet the requirements for clinical diagnostics [3]. Examination of patients with hypo- (53) and hyper- (32) thyroidism, as well as of healthy donors (272) at the I. M. Sechenov Moscow Medical Academy (Laboratory of Hormone Biochemistry, Prof. T. D. Bol'shakova) using our test system and an Amerlite TSH-60 system (Great Britain) demonstrated a high correlation between these two methods (Fig. 1) (the correlation coefficient was 0.96).

We are grateful to E. P. Gitel' and V. A. Kuznetsov (I. M. Sechenov Moscow Medical Academy) for their consultations and assistance in the examination of patients.

REFERENCES

1. L. K. Koryazova, G. I. Kovalevskaya, V. E. Samoilova, and R. G. Vasilov, *Biotekhnologiya,* № 6, 30-32 (1992).
2. E. R. Sigal, L. K. Koryazova, G. I. Kovalevskaya, *et al., Ibid.,* № 3, 31-34 (1993).
3. E. R. Sigal, L. K. Koryazova, G. I. Kovalevskaya, *et al., Ibid.,* № 5, 34-36 (1994).
4. M. Benkirane, D. Bon, M. Cordeil, *et al., Molec. Immunol.,* **24**, № 12, 1309-1315 (1987).
5. M. A. Chlenov, E. I. Kandyba, and Yu. V. Volgin, in: *Proc. 9th International Symposium on Preparative and Industrial Chromatography,* Nancy (1992), pp. 347-352.
6. J. G. Pierce and T. F. Parsons, *Ann. Rev. Biochem.,* **50**, 465-495 (1981).