ELISA TEST SYSTEM WITH MONOCLONAL ANTIBODIES TO HUMAN IgG4 FOR THE DETECTION OF ALLERGEN-SPECIFIC ANTIBODIES IN POLLINOSIS

E. V. Chernokhvostova, T. S. Kotova,
O. I. Atovmyan, E. L. Arsen'eva,
G. T. Bogacheva, and O. V. Rokhlin

UDC 615.373.03:616-056.
3-022.854-078.333

KEY WORDS: ELISA: monoclonal antibodies; IgG4; pollinosis

A world-wide increase in the prevalence of allergic diseases is currently being observed, including one of the most widespread of them, namely pollinosis. The pollinoses are allergic diseases of atopic character, developing on account of the action of pollen from plants. The diagnosis of pollinoses and determination of the responsible allergen are based primarily on clinical data and also on certain skin tests and provocation tests that are by no means free from risk for the patient [2, 14]. Of the immunologic methods in vitro, determination of the level of total immunoglobulin E and of allergen-specific IgE-antibodies is used [3, 11].

It has recently been shown, however, that besides IgE-antibodies, an important role in allergic diseases also is played by IgG4-antibodies [6, 8, 12]. Determination of IgG4-antibodies in atopic allergies is important both to discover the responsible allergen and also to assess the results of hyposensitizing treatment, for the IgG4-antibody level rises in most cases during specific treatment. Thus their role is evidently very important for certain types of food allergies [7, 15].

Determination of IgG4-antibodies can only be done by highly sensitive methods with highly specific reagents. Production of monoclonal antibodies to the human IgG4 subclass and the use of ELISA have made possible the development of a test system to determine IgG4-antibody levels to dust allergens.

EXPERIMENTAL METHOD

Human IgG of various subclasses (IgG1 – 4 proteins, IgG2 – 4 proteins, IgG3 – 4 proteins, IgG4 – 9 proteins) were used; all were isolated by preparative electrophoresis in agarose gel from sera of patients with multiple myeloma [4].

A hybridoma secreting monoclonal antibodies to human IgG4 was obtained by fusion of the splenocytes of a BALB/c mouse immunized with human $IgG4_{\chi}$ Zhel. with mouse plasmacytoma cells of line x63.Ag8.653 [9], by the method of Kohler and Milstein [10]. The hybridoma clones were screened by radioimmunoassay [5]. The selected hybridoma clone was subjected to passage in vivo in the ascites form, and the sulfate fraction of the ascites fluid with a concentration of antibodies of 2 mg/ml was used. Monoclonal rabbit antibodies to mouse IgG, previously adsorbed on human IgG to remove cross-reacting antibodies, also were used.

Sera from patients with pollinosis and with allergy to grass pollen (55 sera) and tree pollen (53 sera) were tested. The control group consisted of sera from 20 healthy blood donors.

G. N. Gabrichevskii Moscow Research Institute of Epidemiology and Microbiology. Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Vorob'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 11, pp. 574-577, November, 1989. Original article submitted January 17, 1989.

The antigens were commercial allergens from the "Allergen" Scientific-Technical Combine. Specific antibodies to a mixture of grass pollen (rye, oats, couch, rye-grass, brome, orchard grass) and to tree pollen (the most widespread allergen) were tested.

Monoclonal antibodies to human IgG4 and rabbit antibodies to mouse IgG were conjugated with horseradish peroxidase by the periodate method [13]. Several different versions of ELISA were used: the specificity and activity of the monoclonal antibodies, not conjugated with peroxidase, to IgG4 was tested in the sandwich version of ELISA, with adsorption of proteins of different human IgG subclasses on a plate in a dose of 10 μg per well; dilutions of antibodies (from 0.03 to 2 μg) were introduced into layer 2, and after incubation and washing, those which had reacted with the first layer of protein were detected with labeled rabbit antibodies to mouse IgG; the specificity and activity of the conjugate of peroxidase with monoclonal antibodies to IgG4 was demonstrated by direct ELISA with adsorption of different subclasses of human IgG and of bovine serum albumin (BSA) on the plate; allergen-specific antibodies of the IgG4 subclass were tested in a four-layer version of ELISA: allergens containing 5000 PNU in 1 ml (1 PNU = 1×10^{-5} mg of protein nitrogen) were applied to the plate in a volume of 0.15 ml; undiluted test sera in a volume of 0.1 ml were introduced in layer 2, and monoclonal antibodies to IgG4 into layer 3, and were subsequently developed with labeled rabbit antibodies to mouse IgG.

In all versions, incubation of layer 1 was carried out overnight at 4°C, and the remaining incubation lasted 1 h at 37°C. Between incubations the plates were washed with buffered physiological saline containing 0.05% Tween-20 solution. Orthophenylenediamine (East Germany) was used as the chromogen and incubation with it continued for 30 min at 4°C. The results of the test were read on a "Multiscan" instrument ("Flow Laboratories"). The level of antibodies was expressed in optical density (OD) units. The plates used in the work were from the Moscow Factory.

EXPERIMENTAL RESULTS

Results of tests of specificity and activity of the monoclonal antibodies to human IgG4, using the 1st and 2nd versions of ELISA (Table 1) show that these monoclonal antibodies react only with proteins of their own isotype. Similar results were obtained with the nine different monoclonal IgG4 and with 12 paraproteins from the other three subclasses of human IgG. The optimal dose (optical density about 1.0 OD units) was 0.03 μg for unlabeled antibodies (version 1). A similar result was obtained by the second version of ELISA with antibodies in a concentration of 4 μg. Thus version 1 of ELISA enables lower concentrations of anti-IgG4-antibodies to be used, and the subsequent work was done with unlabeled antibodies.

Choice of the optimal dose of anti-IgG4 for determination of allergen-specific antibodies of this subclass is shown in Fig. 1, where it can be seen that 0.5 μg of antibodies gives low values, whereas 1 and 2 μg give virtually identical titration curves. In the subsequent work, anti-IgG4 was used in a dose of 1 μg/ml

TABLE 1. Specificity and Activity of Monoclonal Antibodies to Human IgG4

Version of ELISA*	Proteins on plate, 10 μg in well	Concentration of IgG4-antibodies, μg/ml**							
		4	2	1	0,5	0,25	0,125	0,06	0,03
1	IgG4 Br-n	—	>2	>2	>2	>2	1,91	1,41	0,89
	IgG4 Z-ii	—	>2	>2	>2	>2	1,91	1,42	0,84
	IgG4 P-va	—	>2	>2	>2	>2	>2	1,60	1,03
	IgG1 T-ii	—	0,18	0,12	0,09	0,08	0,05	0,05	0,05
	IgG2 K-va	—	0,15	0,13	0,11	0,10	0,07	0,06	0,06
	IgG3 F-ℓ'	—	0,11	0,08	0,06	0,04	0,04	0,04	0,05
2	IgG4 Br-n	1,00	0,66	0,40	0,20	0,12	—	—	—
	IgG1 T-ii	0,02	0,02	0,01	0,0	0,0	—	—	—
	BSA	0,11	0,01	0,01	0,02	0,0	—	—	—

Legend. *) Version 1 – unlabeled monoclonal anti-IgG4 and conjugate of rabbit antibodies to mouse IgG with peroxidase; version 2 – conjugate of monoclonal anti-IgG4 with peroxidase (see: "Experimental Method").
**) Level of reaction expressed by value of optical density at 492 nm; optical dose of antibodies (optical density about 1.0 OD units) was 0.03 μg/ml for version 1 and 4 μg/ml for version 2.

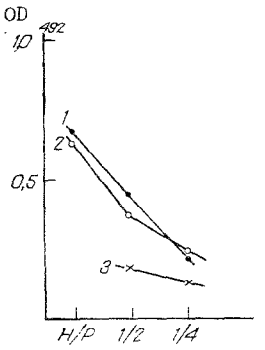

Fig. 1

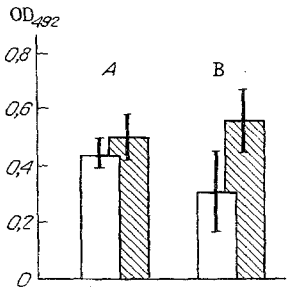

Fig. 2

Fig. 1. Determination of allergen-specific IgG4-antibodies using different concentrations of monoclonal anti-IgG antibodies. Abscissa, dilutions of serum of patient with allergy to birch pollen; monoclonal anti-IgG4-antibodies used in concentration of: 1) 0.5 μg/ml; 2) 1 μg/ml; 3) 2 μg/ml.

Fig. 2. Average levels of allergen-specific IgG4-antibodies in patients with allergy to tree pollen, associated with different intensities of skin tests (a) and different severities of pollinosis (b). a) Shaded columns – strong reactions, unshaded – weak; b) shaded columns – severe form of pollinosis, unshaded – mild form, vertical lines denote confidence interval at a 95% level of probability.

TABLE 2. Mean Level of IgG4-Antibodies to Allergens in Patients with different Types of Pollinosis

Group tested	Number tested	Antibody level ($\bar{X} \pm m^*$)	
		To grass pollen	To tree pollen
Persons with allergy to grass pollen	64	0,08±0,01	0,05±0,01
Persons with allergy to tree pollen	53	0,01±0,0	0,46±0,04
Healthy subjects	20	0,03±0,01	0,04±0,01

Legend. *Optical density.

During investigation of the sera for patients with pollinosis, specific IgG4-antibodies to the responsible allergen were discovered only in patients with allergy to tree pollen and were virtually absent in patients with pollinosis caused by grass pollen and in healthy individuals (Table 2). Determination of IgG4-antibodies in allergy to tree pollen was undoubtedly of diagnostic importance: 81% of positive results in the experimental group compared with only 6% in the control [1].

Evaluation of changes in the level of allergen-specific IgG4-antibodies during hyposensitizing therapy revealed a regular rise of the level of IgG4-antibodies, especially of antibodies to tree pollen allergens. In the untreated patients (32) the mean antibody level was 0.37 ± 0.07, and in the treated patients (21) it was 0.6 ± 0.06 ($p < 0.01$). The results show that determination of allergen-specific IgG4-antibodies can be used to monitor hyposensitizing therapy.

The role of IgG4-antibodies in the pathogenesis of atopic allergy is not clear. In patients with allergy to tree pollen the level of allergen-specific IgG4-antibodies correlated with the severity of the disease, but not with the intensity of the skin tests (Fig. 2). The

intensive IgG4-response in the severe form of pollinosis was evidently due to stronger antigenic stimulation, whereas skin reactions to the allergen were determined not by IgG4-, but by IgE-antibodies. To assess the functional role of IgG4-antibodies it is important to investigate allergen-specific IgG4-antibodies in different types of atopic allergy.

The results of these investigations show that the monoclonal antibodies to human IgG4 which we used are highly specific, react only with proteins of the 4th subclass of IgG, and can be used for the determination of allergen-specific antibodies of this isotype, playing an important and not yet fully studied role in atopic allergy. ELISA determination of allergen-specific antibodies is best used for the diagnosis of allergy to tree pollen and for monitoring specific hyposensitizing therapy.

LITERATURE CITED

1. O. I. Atovmyan, "Nonspecific factors of humoral immunity and allergen-specific IgG, IgE, and IgG4 antibodies in patients with pollinosis," Author's abstract of dissertation for the degree of Candidate of Medical Sciences, Moscow (1988).
2. N. D. Beklemishev, R. K. Ermakova, V. S. Moshkevich, and O. M. Zhukova, Pollinoses: Hay Fever [in Russian], Alma-Ata (1974).
3. V. B. Gervazieva, I. G. Ovsyannikova, N. I. Voronkin, et al., Zh. Mikrobiol., No. 9, 33 (1987).
4. E. V. Chernokhvostova and G. P. German, Immunochemical Diagnosis of Gammapathies: Technical Recommendations [in Russian], Moscow (1984), p. 7.
5. V. V. Cherepakhin, A. V. Chervonsky, A. V. Filatov, and O. V. Rokhlin, Immunol. Lett., 10, No. 2, 217 (1985).
6. R. Djurup, Allergy, 40, No. 1, 469 (1985).
7. S. Breborg and T. Foucard, Allergy, 38, No. 3, 167 (1983).
8. M. Etievant, B. Leluc, R. Bouclier, and E. Lecocq, Ann. Allergy., 43, No. 3, 169 (1979).
9. J. F. Kearney, A. Radbruch, B. Liesegang, and K. Rajewsky, J. Immunol., 123, No. 4, 1548 (1979).
10. G. Kohler and C. Milstein, Nature, 256, 495 (1975).
11. J. McDonnell, H. C. Doner, B. Martin, and H. S. Nelson, J. Allergy, 63, No. 3, 202 (1979).
12. T. Nakagawa, Monogr. Allergy, 19, 253 (1986).
13. P. K. Nakane and A. Kawaoi, J. Histochem. Cytochem., 22, 1084 (1974).
14. J. Ring, Behring Inst. Mitt., 68, 141 (1981).
15. F. Shakib, H. M. Brown, and D. R. Stanworth, Int. Arch. Allergy, 75, No. 2, 107 (1984).