

Isolation and Characterization of Monospecific Antibodies to β_2 -Microglobulin

I. V. Shabunina, O. I. Afanas'eva, and S. N. Pokrovskii

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Monospecific polyclonal antibodies were isolated from sheep antiserum to β_2 -microglobulin by affinity chromatography and ammonium sulfate fractionation followed by ion-exchange chromatography. Antibodies were characterized by polyacrylamide gel electrophoresis, immunoblotting, and double radial immunodiffusion according to Ouchterlony. The isolated antibodies are highly specific and can be used for measuring β_2 -microglobulin in human serum by Mancini's radial immunodiffusion method.

Key Words: β_2 -microglobulin; immunodiffusion; chronic renal insufficiency

β_2 -Microglobulin (β_2 -M), a protein with molecular weight of about 12 kD, is found on the plasma membrane of almost all eukaryotic cells as the light chain of a major histocompatibility complex class I antigen [7]. In patients on chronic hemodialysis serum concentration of β_2 -M increases ten times and higher in comparison with the norm (1.2-2.7 mg/liter) [1], which is the cause of amyloidosis [2]. The development of simple methods for measuring serum β_2 -M concentration and for decreasing it is a pressing task in the treatment of patients with chronic renal insufficiency. Our purpose was to isolate and characterize monospecific polyclonal antibodies to β_2 -M and to study the possibility of their use in clinical practice.

MATERIALS AND METHODS

For preparing monospecific antisera, sheeps were intramuscularly injected with β_2 -M (GLT Biotrack Inc.) in a half-dose of 0.5 mg protein/animal in phosphate buffer (pH 7.4) with complete Freund's adjuvant (CFA). Reimmunization was carried out after 7 weeks by an intramuscular injection of complete dose of the antigen (1 mg) with CFA. Blood for analysis was collected on day 10 after antigen injection. Then reim-

munizations were carried out every 1.5 months with complete dose of the antigen with incomplete Freund's adjuvant, and blood was collected from donor animals on days 10, 12, and 14 after injection of the antigen. NaN_3 was added to the serum to a final concentration of 0.02%. The sera were frozen and stored at -20°C .

The concentration of antibodies to β_2 -M was measured by enzyme-linked immunosorbent assay (ELISA). To this end, β_2 -M was incubated in Librio plates (1 μg protein/well) for 16 h at 4°C . Test antisera diluted 1:10 in phosphate buffer and titrated by serial 1:5 dilutions were added to the plates. Antibodies to β_2 -M in a concentration of 0.5 mg/ml served as the standart. Then rabbit antibodies to sheep IgG conjugated with peroxidase (Sigma) were added. The enzymatic reaction of orthophenylene diamine staining in 0.2 M citrate buffer (pH 4.5) was stopped by adding 50% sulfuric acid. Measurements were carried out on a Titertek Multiscan (Flow) at 492 nm. The concentration of antibodies in donor animal sera was estimated from the calibration curve.

Anti- β_2 -M IgG fraction of the antiserum was isolated by fractionation with $(\text{NH}_4)_2\text{SO}_4$ (final concentration 25%). The precipitate was separated by centrifugation at 3000g for 30 min and dialyzed against 0.1 M borate buffer (pH 8.1), after which ion-exchange chromatography on DE-52 cellulose (Whatman) was carried out. In addition, antibodies were isolated from antisera by affinity chromatography on

Institute of Experimental Cardiology, Russian Cardiology Research-and-Production Complex, Ministry of Health of the Russian Federation, Moscow

a sorbent containing β_2 -M as the ligand. β_2 -M was immobilized on CL-4B Sepharose (Pharmacia) activated with cyanogen bromide (0.5 mg/ml gel) [6]. The column was equilibrated with phosphate buffer, and antibodies were eluted with 0.05 M citrate buffer, pH 2.5. The antibodies were investigated by vertical electrophoresis in 10% polyacrylamide gel under denaturing conditions in the presence of SH reagent [3].

The specificity of antibodies was evaluated by Ouchterlony's radial immunodiffusion method [5] and by immunoblotting with β_2 -M preparations, β_2 -M-rich fraction (lyophilized urine of patients with chronic renal insufficiency), and plasma of patients on chronic hemodialysis.

Proteins were separated by gradient electrophoresis in 5-22% polyacrylamide gel in the presence of SDS, transferred to nitrocellulose at 30 mA current for 16 h at 4°C in a buffer containing 20% ethanol, 0.19% Tris (hydroxymethyl)aminomethane, and 0.9% glycine. Nitrocellulose was incubated for 30 min in 1% solution of sheep albumin in phosphate buffer and then 2 h with test antibodies to β_2 -M conjugated with horseradish peroxidase, using 2-chloronaphthol as the substrate.

RESULTS

ELISA showed that the concentration of antibodies to β_2 -M in antisera increases after the first 3 immunizations, reaching a plateau by the fourth injection (Fig. 1). This confirmed the adequacy of the immunization protocol used. Antiserum specificity was tested by immunodiffusion method according to Ouchterlony: the antiserum reacted with β_2 -M and yielded a single precipitation band with β_2 -M-rich fraction (Fig. 2).

The antiserum was used for isolation of monospecific polyclonal antibodies to human β_2 -M. The IgG-containing fraction was isolated by sulfate fractionation; the fraction was then purified by ion-exchange chromatography on DE-52 cellulose (protein yield 53%). Vertical electrophoresis under denaturing conditions in the presence of SH reagent showed that the isolated protein is highly purified IgG (Fig. 3). Electrophoregrams showed 2 major bands corresponding to heavy and light chains of the IgG molecule (50 and 25 kD, respectively). Double radial immunodiffusion according to Ouchterlony showed that these antibodies are specific to β_2 -M and do not react with human albumin and immunoglobulins. The obtained monospecific polyclonal antibodies to β_2 -M were used to develop a quantitative immunochemical test-system for measuring β_2 -M in human plasma using Mancini's radial immunodiffusion method [4]. During reaction of β_2 -M with antibodies polymerized in agarose gel, a precipitate ring formed with a square diameter direct-

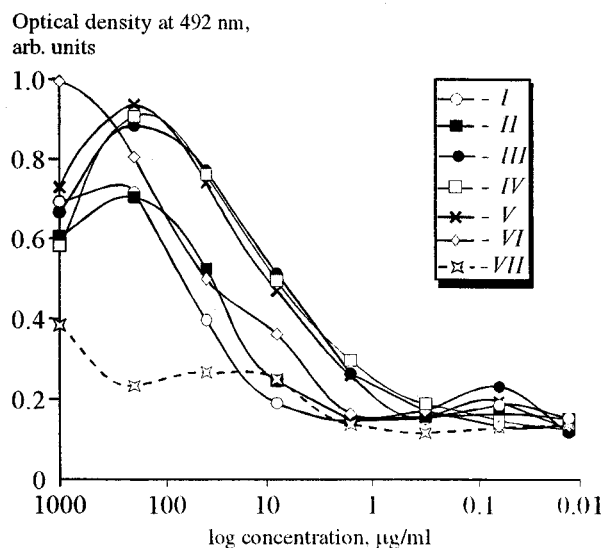


Fig. 1. Relationship between optical density at 492 nm and the concentration of antibodies to β_2 -microglobulin in sheep antiserum in enzyme immunoassay. I-V) 1st-5th immunizations, respectively; VI) control (positive control with antibody solution in a concentration of 0.5 mg/ml); VII) nonimmune serum.

ly proportional to β_2 -M concentration in the plasma. This β_2 -M assay can be used as a screening test in laboratory studies and for creating a simple and cheap diagnostic system.

ELISA showed that sulfate fraction of the antiserum contains only 10% antibodies specific for β_2 -M, while the rest 90% were IgG not specific to β_2 -M and other proteins precipitated with ammonium sulfate. Highly purified antibodies were isolated by affinity chromatography on a sorbent containing human β_2 -M as the ligand (the yield was 53 mg protein from 50 ml serum). Electrophoresis of the eluate (Fig. 3) showed that the resultant preparation contained IgG alone and was free from other proteins. Electrophoresis showed only heavy and light IgG chains.

The specificity of the resultant antibodies was evaluated by Ouchterlony's radial immunodiffusion method. The presence of a single precipitation band

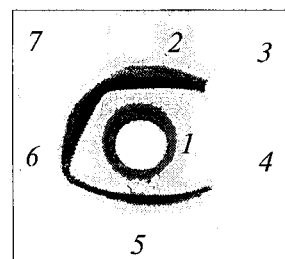


Fig. 2. Double radial immunodiffusion according to Ouchterlony. Wells contained 15 μ l sheep antiserum (1), β_2 -microglobulin isolated from plasma by affinity chromatography (2), human IgG (3), human serum albumin (4), β_2 -microglobulin in phosphate buffer (5), fraction depleted of (6) or rich in β_2 -microglobulin (7).

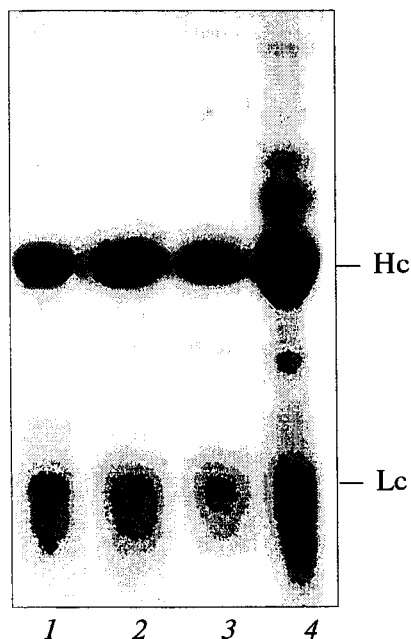


Fig. 3. Electrophoresis under denaturing conditions in the presence of SH reagent. 1) antibodies isolated by affinity chromatography; 2) IgG fraction after purification on DE-52 cellulose; 3) non-adsorbing fraction after ion-exchange chromatography; 4) antiserum protein fraction after precipitation with ammonium sulfate. Hc and Lc: heavy and light chains of IgG, respectively.

with β_2 -M and an identical band with β_2 -M-rich fraction and the absence of any other precipitation bands confirm monospecificity of the isolated antibodies. β_2 -M, human plasma, and β_2 -M-rich fraction were tested. The bands of antibody reaction with β_2 -M-rich fraction on nitrocellulose plate were identical to the bands of antigen-antibody reaction. Single band with

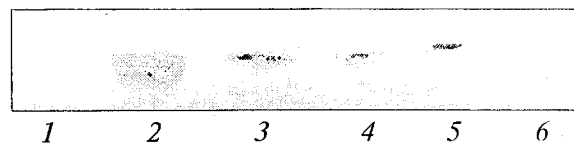


Fig. 4. Immunoblotting with antibodies to β_2 -microglobulin (β_2 -M). 1, 6) β_2 -M; 2) β_2 -M-rich fraction; 3-5) sera from patients on chronic hemodialysis.

human plasma confirmed a high specificity of the isolated antibodies (Fig. 4).

Hence, monospecific polyclonal sheep antibodies to human β_2 -M were isolated and characterized. A method of radial immunodiffusion with these antibodies was developed for measuring β_2 -M in the plasma in a concentration range of 2-12 mg/liter. For β_2 -M concentrations of 4-12 mg/liter, the coefficient of variations between measurements was 3.0-7.1% within a series and 13% between series; at low concentrations (1.6-3 mg/liter) it was higher: 21% within a series and 35% between series.

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