

Development and characterization of polyclonal antibody against human kappa light chain in rabbit

Mojgan Esparvarinha^{1,2,3}, Hamid Nickho^{1,2}, Leili Aghebati-Maleki^{1,2,3*}, Jalal Abdolalizadeh⁴, Hadi Nasiri^{1,2}, Zahra Valedkarimi^{1,2}, Jafar Majidi^{1,2*}

¹Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; ²Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran; ³Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran; ⁴Immunology Laboratory, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

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Abstract

Polyclonal antibodies against kappa light chain are used to diagnose diseases producing free light chain. The kappa and lambda light chains are products of immunoglobulin synthesis and released into the circulation in minor amounts such as serum, cerebrospinal fluid, urine and synovial fluid in normal condition. The purpose of this study was the production and purification of polyclonal immunoglobulin G (IgG) against human kappa light chains. In this study, early human IgG was purified by ion-exchange chromatography, reduced with Dithiothreitol and heavy and light chains were separated with size-exclusion chromatography. Afterward, affinity chromatography with protein L Sepharose at pH 2.00 was displayed to be a dominant condition for the separation and purification of the kappa light chain of immunoglobulins from human serum. Eventually, the rabbit was immunized by human kappa light chains. The rabbit IgG was purified and labeled with horseradish peroxidase (HRP). Direct enzyme-linked immunosorbent assay was planned to determine the titer of HRP conjugated rabbit IgG against the human kappa light chain. The optimum titer of anti-kappa IgG was 1:16000. At the result, purified polyclonal anti-kappa is useful tool in biomedical and biochemical researches and diagnostic kits.

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Introduction

There are firm evidences showing that immunoglobulins are widely involved during host immune responses. The immunoglobulins contain two equal heavy chains and two equal light chains connected via disulfide bonds to form tetrameric structures. In the mammalian immune system, the light chains are comprised of two subtypes, κ or λ . In humans, each plasma cell creates only one chain type. Interestingly, the genes encoding κ and λ chains are organized on different chromosomes (chromosomes 2 and 22, respectively).^{1,2} In healthy people, polyclonal (κ and λ) and free light chains (FLCs) are produced in large quantities; however, their accurate function is unclear. It is known that plasma cell neoplasms

secrete only κ or λ light chains. In normal conditions, approximately 500 mg of FLCs are produced per day in a lymphoid system which are subsequently secreted and catabolized through the kidney.^{3,4} These chains are products of immunoglobulin synthesis and released, in the minor amounts, into the circulation such as serum, cerebrospinal fluid, urine and synovial fluid in normal condition.⁵

Bence Jones (BJ) proteins are homogeneous populations of κ or λ molecules produced by malignant clones of B cells.⁶ One principally useful property of serum FLCs is their short half-life in the blood (λ : 2 - 4 hr; κ : 3 - 6 hr) in contrast to complete immunoglobulin (21 days), which provides an occasion for real-time monitoring of disease progress and answer to treatment.⁷ In myeloma, increased levels of monoclonal FLC are noticeable as a consequence

*Correspondence:

Leili Aghebati-Maleki. PhD

Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

E-mail: aghebatil@tbzmed.ac.ir

Jafar Majidi. PhD

Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

E-mail: majidij@tbzmed.ac.ir



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of the clonal expansion of plasma cells and additionally, increased concentration of oligoclonal/polyclonal FLC due to the increased immune production or decreased clearance including autoimmune/inflammatory diseases and plasma cell proliferative disorders.⁸⁻¹³ An increased FLC concentration has also been linked to diseases relapse and stimulated immune responses, suggesting a strong contribution of FLCs in the chronic state of inflammation.⁶ Evaluation of FLCs molecular weight was performed by size-exclusion chromatography on Sephadex G100¹⁴ which will be further explained in the present study. The purpose of this study was to produce antibodies against human kappa light chain in the rabbits. In the current study, we aimed to produce cost-effective IgG against kappa light chain with high affinity to be used as a diagnostic agent.

Materials and Methods

Human immunoglobulin G (IgG) purification. Blood samples were taken from patients referred to the Imam Reza Hospital (Tabriz, Iran) with their informed consent (No. TBZMED.REC.1394.457). Peripheral blood samples were prepared by centrifugation (1,000 *g* and 15 min) and then diluted 1:1 with phosphate buffer saline (PBS; Sigma, Philadelphia, USA) at pH 7.40 for IgG purification. The precipitation was done at 4 °C that equal volumes of diluted serum and saturated ammonium sulfate were blended through the slow addition of ammonium sulfate solution during mild stirring. On the next day, this sample was centrifuged (3,500 *g* for 20 min) and subsequently washed twice with 50.00% saturated ammonium sulfate solution (Sigma). The precipitate was liquefied in PBS and then dialyzed against PBS. The ending solution was filtered via a 0.22 µm Millipore filter (Bio-Rad, Hercules, USA), and the clear supernatant was loaded onto the column. Ion-exchange chromatography was done on a DEAE-Sepharose that column was equilibrated with Tris-HCl buffer (Abcam, Cambridge, UK) at a flow rate of 1.00 - 2.00 mL per min. The sample was loaded onto the column and eluted with Tris-HCl buffer. The first peak was purified IgG. The purity of several IgG preparations was tested through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Free κ and λ molecules were prepared by reduction and alkylation of polyclonal IgG. Polyclonal IgG was dissolved in 3.00 mL of 8.00 M deionized urea and 0.60 M Tris-HCl with pH 8.60. Subsequently, 5.00 µL dithiothreitol (0.50 M) was added and it was incubated for 3 hr at room temperature. After 3 hr, 0.30 mL of iodo-acetamide was added and incubated in the dark for 30 min at 37 °C. Alkylated FLCs were purified by Sephadex G-100 (Pharmacia, Uppsala, Sweden) column. When human IgG was reduced, heavy and light chains were separated with a gel-filtration. Gel-filtration was performed using the Sephadex G-100 column in 0.10 M Tris buffer with pH 7.50. The columns were equilibrated

with 100 mL of Tris buffer (0.10 M) in pH 7.50, afterward, the sample was loaded at a 1.00 - 1.50 mL per min flow rate. Elution of FLCs from the column was monitored with ultraviolet absorption at 280 nm. The FLC purity was evaluated using SDS-PAGE.

Separation κ and λ molecules with protein L.

Affinity chromatography was performed for isolating kappa light chain at room temperature column coupled to protein L. The column was equilibrated with PBS in pH 7.40 at a flow rate of 1.00 mL per min. After sample application, the column was rinsed with PBS, until the absorbance approached baseline. The bound human κ light chain was eluted with 0.10 M glycine-HCl (Sigma) with pH 2.00. The absorbance of fractions was measured at 280 nm. Acidic fractions were immediately neutralized with 1 M Tris at pH 7.50 - 9.00.

Immunization protocol and screening of immunized rabbit.

Antibody production was performed on a seven-month-old New Zealand white rabbit. These procedures were done according to the Animal Laboratory Guidelines and approved by the Regional Medical Sciences Research Ethics Committee of Tabriz University of Medical Sciences (No. TBZMED.REC.1394.457). Rabbit received antigen in four steps. The first injection was done by 300 µg per 300 µL of kappa light chain (Sigma, Deisenhofen, Germany) with the same volume of Freund's complete adjuvant (Sigma). Immunization was followed by two boosters. Inoculations of antigen in a Freund's incomplete adjuvant (Sigma) emulsion was administered intramuscularly on days 22 and 36. Final immunization was done without any adjuvant on day 60. After each immunization, the rabbit was monitored daily for any side effects.

Rabbit anti-IgG kappa light chain purification.

Rabbit anti-IgG kappa light chain was purified by ion-exchange chromatography method on a DEAE-Sepharose column (Pharmacia). The column was equilibrated by 40 mM Tris-phosphate buffer at pH 8.10. The IgG was eluted by 75 mM Tris-HCl buffer at pH 8.10. Purity was assessed by SDS-PAGE and purified antibodies were visualized in the gel by Coomassie brilliant blue G-250 staining.

Enzyme-linked immunosorbent assay (ELISA).

Conjugation of horseradish peroxidase (HRP) to rabbit IgG was carried out using periodate oxidation method. Anti-rabbit IgG-HRP conjugate was primed and used for ELISA experiment.¹⁵ Direct ELISA was planned to determine the titer of HRP conjugated rabbit IgG against human kappa light chain. The κ light chain (100 µL) was coated onto each well of a well and the plate was incubated at 4 °C for 24 hr. After incubation, the extra antigen was removed from the well by washing three times with PBS-Tween (0.05% Tween 20; Merck, Darmstadt, Germany) and non-specific sites were blocked with blocking solution (PBS-0.50% Tween 20). After washing, 100 µL of 1:500, 1:1000, 1:2000, 1:4000, 1:8000, 1:16000 1:32000 and 1:64000 dilutions of primed HRP

conjugated anti-human kappa were added to each well and the plate was incubated for 30 min at 37 °C. Subsequently, 100 μ L of the chromogenic substrate, tetra-methyl-benzidine, was added to each well and the plate was incubated for 10 min at room temperature till color developed. Developed color was read at 450 nm.¹⁵

Results

The IgG was purified from serum through a simple one-step ion-exchange chromatography technique. Twenty-five mg protein was loaded on the ion exchange chromatography column. Twelve mg human IgG antibody was yielded in purification by ion-exchange chromatography. The IgG-rich fractions attained were examined for purity via SDS-PAGE. The purity of the fraction was up to 95.00%. The reduced IgG was isolated by gel-filtration. First, non-reduced IgG is eluted, then heavy chain and at the end light chains. The non-reducing SDS-PAGE has been run after gel-filtration, which the results showed that the mass of light chain (LC) and heavy chain (HC) is only about 25.00 and 50.00 KDa, respectively (Figs. 1 and 2A). Affinity chromatography with protein L sepharose at pH 2.00 was displayed to be a dominant technique for the separation and purification of kappa light chain of immunoglobulins from human serum. About 5.00 mg of protein was obtained using this method.

Highly pure fraction was performed as pure bands in SDS-PAGE analysis (Fig. 2B). Direct ELISA was used to determine the optimal titer of conjugated IgG against human kappa produced in the rabbit and the titer was about 1:16000.

The kappa light chain was injected with Freund's adjuvant. The rabbit serum at 1:64000 dilution showed high absorbance in reaction with injected antigen (κ chain) at the designed ELISA method. The Fig. 3 shows the results of the purification of polyclonal antibody against kappa chain in rabbit serum.

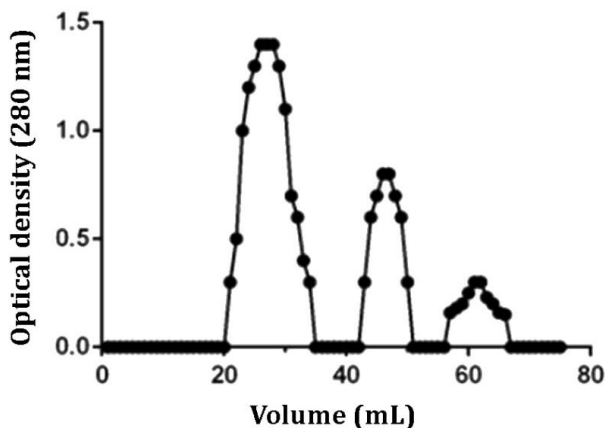


Fig. 1. Gel-filtration fractionation of IgG light chain and heavy chain and unreduced IgG. Gel-filtration was done using a Sephadex G-100 column with 0.10 M Tris buffer at pH 7.50.

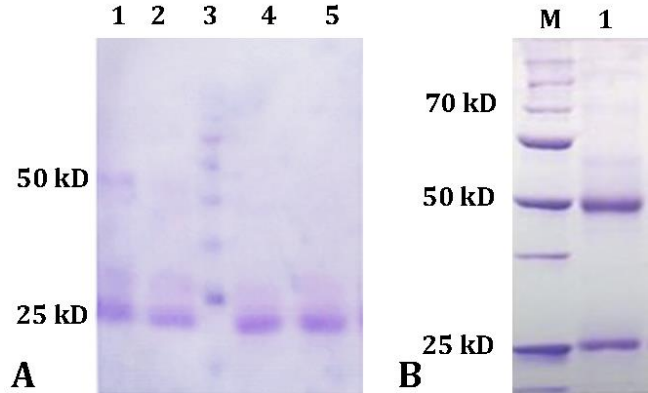


Fig. 2. **A)** The non-reducing SDS-PAGE has been run after the gel-filtration. Lanes 1 and 2: Heavy chain with light chain contaminant; Lane 3: Ladder; Lanes 4 and 5: Light chain; OD: 0.3; **B)** SDS-PAGE analysis of rabbit polyclonal antibody for purity determination of polyclonal anti-kappa. Lane 1: Reduced anti-kappa; M: Marker.

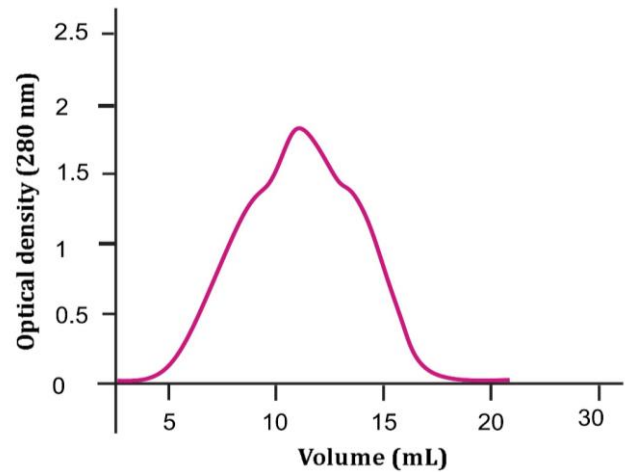


Fig. 3. Purification of rabbit polyclonal anti-kappa IgG by ion-exchange chromatography. Isolation of polyclonal anti-kappa IgG by 75 mM Tris-HCl buffer at pH 8.1.

Discussion

The therapeutic and diagnostic targets for antibody fragments are important, their numerous structural formats cause struggles for production and purification. The FLCs provide an occasion for real-time monitoring of disease development and response to treatment such as HIV infection and non-Hodgkin's lymphoma (NHL). Evaluation of FLC can be used as a marker for B cell activation and could be useful for identifying HIV-infected individuals at increased NHL risk.^{16,17} It has been reported that about 60.00% of κ -type light chains are comprised κ I, κ III and κ IV proteins.^{18,19}

In this study, the kappa light chain protein was isolated with protein L. In order to study antibody production in rabbits and immunization estimation, ELISA test was performed. Obtained polyclonal antibody titer (1:16000)

could be beneficial for many types of detection methods and shows the high quality of the product. The SDS-PAGE analysis exhibited the protein with approximately 25.00 kDa and the titer of direct ELISA was 1:16000. We showed that the low molecular weight of 25.00 kD of light chain can produce optimal titer antibodies. A noticeable amount of anti-IgG antibody could be obtained, which would meet many types of research and educational medical requirements. Produced polyclonal anti-kappa light chain antibody can be used in designing of ELISA kits.

Increased FLC levels can be related to disease relapses, immune system higher activity, and disease progression. Estimating polyclonal FLCs as an indicator of B cell activation can disclose the activity of the adaptive immune system in a diverse variety such as inflammatory diseases, lupus Erymanthos, inflammatory bowel disease and other inflammatory conditions.¹

Purified antibody against kappa light chain can be used as an evaluation and immunogenic factor of polyclonal immune serum. This product is highly specific and functional in the biomedical research and diagnostic applications such as immunoassay tests including epitope-mapping, immunofluorescence ELISA, immunohistochemistry, immuno-electrophoresis, western blot, flow cytometry, and WBC X match tests. Anti-kappa light chains are used widely all over the world as diagnostic tools in medicine for inflammatory diseases patients and for treatment of chemotherapy receiving patients.¹² This product is suitable for use as a primary reagent in enzyme immunoassays, western blot and cell or tissue immunostaining.

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Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Esparvarinha M, Nickho H, Mohammadi H, et al. The role of free kappa and lambda light chains in the pathogenesis and treatment of inflammatory diseases. *Biomed Pharmacother* 2017; 91: 632-644.
2. van der Heijden M, Kraneveld A, Redegeld F. Free immunoglobulin light chains as target in the treatment of chronic inflammatory diseases. *Eur J Pharmacol* 2006; 533(1-3): 319-326.
3. Nasiri H, Valedkarimi Z, Aghebati-Maleki L, et al. Production and purification of polyclonal antibody against F (ab')₂ fragment of human immunoglobulin G. *Vet Res Forum* 2017; 8 (4) 307-312.
4. Abe M, Goto T, Kosaka M, et al. Differences in kappa to lambda (kappa: lambda) ratios of serum and urinary free light chains. *Clin Exp Immunol* 1998; 111(2): 457-462.
5. Valedkarimi Z, Nasiri H, Aghebati-Maleki L, et al. Production and characterization of anti-human IgG F (ab')₂ antibody fragment. *Hum antibodies* 2018; 26(4): 171-176.
6. Sinclair D, Dagg J, Smith J, et al. The incidence and possible relevance of Bence-Jones protein in the sera of patients with multiple myeloma. *Br J Haematol* 1986; 62(4): 689-694.
7. Davids MS, Murali MR, Kuter DJ. Serum free light chain analysis. *Am J Hematol* 2010; 85(10): 787-790.
8. Goffette S, Schlupe M, Henry H, et al. Detection of oligoclonal free kappa chains in the absence of oligoclonal IgG in the CSF of patients with suspected multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2004; 75(2): 308-310.
9. Hopper JE, Sequeira W, Martellotto J, et al. Clinical relapse in systemic lupus erythematosus: Correlation with antecedent elevation of urinary free light-chain immunoglobulin. *J Allergy Clin Immunol* 1989; 9(4): 338-350.
10. Campbell JP, Cobbold M, Wang Y, et al. Development of a highly-sensitive multi-plex assay using monoclonal antibodies for the simultaneous measurement of kappa and lambda immunoglobulin free light chains in serum and urine. *J Immunol Methods* 2013; 391(1-2): 1-13.
11. Dispenzieri A, Kyle R, Merlini G, et al. International myeloma working group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. *Leukemia* 2009; 23(2): 215-224.
12. Hutchison CA, Harding S, Hewins P, et al. Quantitative assessment of serum and urinary polyclonal free light chains in patients with chronic kidney disease. *Clin J Am Soc Nephrol* 2008; 3(6): 1684-1690.
13. Sadeghi S, Aghebati-Maleki L, Nozari S, et al. A methodological approach for production and purification of polyclonal antibody against dog IgG. *Vet Res Forum* 2018; 9 (1) 13-18
14. Drayson M, Tang LX, Drew R, et al. Serum free light-chain measurements for identifying and monitoring patients with nonsecretory multiple myeloma. *Blood* 2001; 97(9): 2900-2902.
15. Maleki LA, Majidi J, Baradaran B, et al. Production and characterization of murine monoclonal antibody against synthetic peptide of CD34. *Hum Antibodies* 2013; 22(1-2): 1-8.
16. Zemlin AE, Ipp H, Rensburg MA, et al. Serum free light chains in patients with HIV infection: Their association with markers of disease severity and antiretroviral use. *J Clin Pathol* 2015; 68(2):148-153.

17. Bibas M, Trotta MP, Cozzi-Lepri A, et al. Role of serum free light chains in predicting HIV-associated non-Hodgkin lymphoma and Hodgkin's lymphoma and its correlation with antiretroviral therapy. *Am J Hematol* 2012; 87(8): 749-753.
18. Nilson B, Solomon A, Bjorck L, et al. Protein L from *Peptostreptococcus magnus* binds to the kappa light chain variable domain. *J Biol Chem* 1992; 267(4): 2234-2239.
19. Liu H, Gaza-Bulsecu G, Chumsae C. Analysis of reduced monoclonal antibodies using size exclusion chromatography coupled with mass spectrometry. *Am Soc Mass Spectrom* 2009; 20(12): 2258-2264.