SHORT COMMUNICATION

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Indigenous production, characterization and evaluation of polyclonal antibody against *Camelus dromedarius* immunoglobulin

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Abstract

No diagnostic kits and reagents are available in the market to detect and evaluate camel immune responses to different pathogens. This study aimed to produce sheep anti-camel (*Camelus dromedarius*) polyclonal antibodies (pAbs) and to determine the specificity with other species immunoglobulin. Immunoglobulins (Igs) from camel serum samples were purified using ammonium sulfate precipitation (40.00% saturated ammonium sulfate). Purity of the camel Igs was tested by sodium dodecyl sulfate polyacrylamide gel electrophoresis. PAbs against (*Camelus dromedarius*) immunoglobulins were generated by immunizing sheep with purified Igs. Anti- camel Ig polyclonal antibodies titer and specificity were determined using ELISA and Western blot techniques. Polyclonal antibodies specific to camel Igs were significantly high in immunized sheep which confirmed the immunization procedure. PAbs reacted specifically with camel serum immunoglobulin and did not react with other species immunoglobulin of horse and chickens. Polyclonal antibodies produced in this study can be regarded as a valuable tool to be used for immune-diagnostic purposes in camel population world-wide.

Introduction

For most mammals, IgG is a large (160 kDa) protein consisting of two identical heavy (H-chain) and two identical light (L-chains) polypeptides, which is highly conserved in mammals.[1] Camels produce three immunoglobulin sub classes of IgG (IgG_1, IgG_2 and IgG_3) in their serum.[2-4] In camels, IgG_2 and IgG_3 subclasses consist of only two heavy chains having molecular weights of 110 kDa as compared to subclass IgG_1 with 160 kDa.[5] Serological techniques are simple, inexpensive, convenient and rapid for diagnosis in many infectious diseases. Diagnosis requires specific reagents for accuracy and sensitivity. However, monoclonal antibodies (mAbs) as reagent are highly specific and sensitive but polyclonal antibodies (pAbs) offer advantage of being cost-effective and easier to produce. Polyclonal antibodies using different labels (fluorescent dyes and enzymes) have been used in immunoblotting and enzyme linked immuno-sorbent assay (ELISA) techniques for diagnosis of camel diseases.[6] Better understanding of camel immune system and immune responses is needed to design strategies for the prevention of infectious diseases. In this study diagnostic reagents were produced to facilitate accurate diagnosis and monitoring of immune responses for the control of infectious diseases, which adversely affecting camel productivity. Polyclonal anti-camel IgG antibodies raised in sheep were tested for specificity against IgGs of camel (*Camelus dromedarius*) and other species.

Materials and Methods

Serum immunoglobulin (Ig) preparation. Blood samples were collected from clinically healthy camel using sterilized disposable needles. Blood was centrifuged at 1,000 *g* for 15 min and the pooled sera were collected and gently mixed with equal volume of 40.00% saturated ammonium sulfate. The mixture was left to stand over-night at 4.00˚C and was centrifuged at 9,000 *g* for 15 min at 4.00˚C. Supernatant was removed

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and pellets were resuspended in sterilized phosphate-buffered saline (PBS) (Merck, Darmstadt, Germany) and dialyzed against PBS at 4.00˚C overnight. Dialyzed samples were stored at –20.00˚C and purity and molecular weight of isolated Ig were analyzed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 12.00% resolving and 5.00% stacking acrylamide gel.[7] Concentration of the purified IgG was determined by Bradford method using bovine serum albumin (BSA) as standard.[8]

Sheep anti-camel Ig polyclonal antibody production. For immunization, 1.00 mL of purified camel IgG (1.00 mg dose 1.00 mL^{-1}) was emulsified with equal volume of Freund's complete adjuvant (Sigma Aldrich, St Louis, MO, USA) in the first immunization wherein 1.00 mL was injected subcutaneously in the sheep. From second to fifth immunization, Freund's complete adjuvant was replaced with the Freund's incomplete adjuvant. All immunizations were carried out at weekly interval. Control group was administered with 1.00 mL of sterilized PBS with 1.00 mL adjuvant. Blood samples were collected one day before each immunization and one week after the last immunization.[9]

Anti-camel Ig polyclonal antibody ELISA. Anti-camel Ig polyclonal antibody titers and specificity were determined using ELISA. Microtiter plates with 96 wells were coated in duplicate with 100 µL - well of purified camel Ig (0.10 µg mL^{-1} in 50.00 mM carbonate / bicarbonate buffer pH 9.60) and incubated at 4.00 ˚C overnight. Uncoated surfaces were blocked using 200 µL - well of blocking solution (PBS at pH 7.20 with 5.00 % skimmed milk) (Merck, Darmstadt, Germany) and incubated for 2 hr at 37.00 ˚C. Plates were washed five times with washing buffer PBST (PBS at pH 7.20 with 0.05% Tween 20) (Merck) and incubated with 100 µL - well of sheep anti-camel Ig polyclonal antibody diluted 50 times with (PBS at pH 7.20 with 1.00% bovine serum albumin) at 37.00˚C for 1 hr. Plates were washed five times with PBST and incubated for 1 hr with 100 µL - well of horseradish peroxidase conjugated donkey-anti-sheep IgG (Sigma-Aldrich) diluted 1 : 6,000. Plates were washed five additional times and a color reaction was initiated by adding 100 µL-well of O-Phenylene diamine dihydrochloride (OPD) (Sigma Aldrich, St. Louis, USA) substrate and incubated at room temperature for 15 min. Optical density (OD) of each well was determined at 450 nm with microtiter plate reader (Immunoskan BDSL; Thermo Lab Systems, Helsinki, Finland).

Cross reactivity of sheep anti-camel Ig antibody to Ig of other animals using Western blotting. Cross-reactivity and specificity of the anti-camel Ig antibody were also evaluated using Western blot analysis. Purified animal species' Ig of camel, horse and chicken were electrophoresed on SDS-PAGE polyacrylamide gel using 12.00% resolving and 5.00% stacking gels; protein bands were transferred to nitrocellulose membrane using semi-dry blotting. Membranes were blocked with TBS (Tris-buffered saline) containing 3.00% skimmed milk (Merck) and incubated at room temperature (RT) overnight; the membranes were washed 3 times with TBST (TBS at pH 7.20 with 0.05% Tween 20; Merck). Blotting membranes were incubated individually with either diluted 50 times with blocking buffer for 1 hr at RT. The Membranes were washed 3 times with TBST and incubated with horseradish peroxidase-conjugated donkey anti-sheep IgG diluted 1 : 1,000 for 1 hr at RT. Finally, membranes were visualized by staining with 0.05% diaminobenzidine (DAB; Sigma Aldrich) in 50.00 mM Tris pH 7.40 containing H_2O_2.

Statistical analysis. Data was analyzed by one-way analysis of variance (ANOVA) in SPSS Software (version 16.0; SPSS, Inc., Chicago, USA). The results were given as mean ± standard deviation and differences were considered significant when $p < 0.05$.

Results

This study endeavored to produce polyclonal antibodies specific to camel Ig in sheep. SDS-PAGE analysis of purified camel Ig revealed purity of the products at about 26.00 kDa as a light chain and 66.00 kDa, 45.00 kDa, and 50.00 kDa as a heavy chain (Fig. 1). Reactivity and specificity of the sheep anti-camel Ig antibody were determined using ELISA and Western blot analysis. Specific antibody against camel Ig resulted from the second week after first immunization (Fig. 2). Anti-camel Ig antibodies were detected in sheep and were significantly higher than the control group ($p < 0.05$). Antibody responses increased from day 7 to 42 and peaked at day 42, following fifth immunization. In Western blot analysis, cross-reaction analysis of sheep anti-camel Ig antibody showed high reactivity with camel Ig and no cross reactivity with horse and chicken Ig.

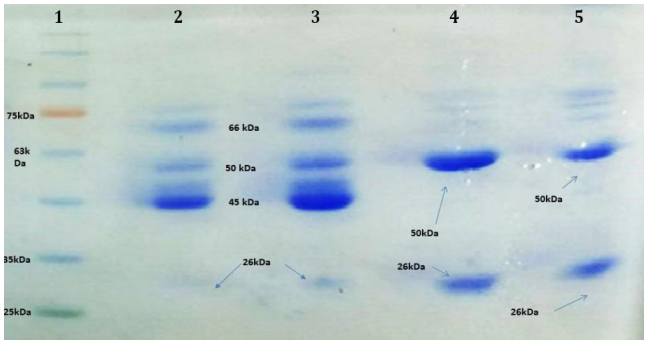

Fig. 1. SDS-PAGE analysis of camel immunoglobulin indicating the location of immunoglobulin H and L chains. Lane 1: Size of molecular weight marker (Cinnagen; [SL7012] prestained protein ladder, Tehran, Iran). Lanes 2 and 3: Purified camel immunoglobulin. Lanes 4 and 5: purified horse and chicken immunoglobulin.

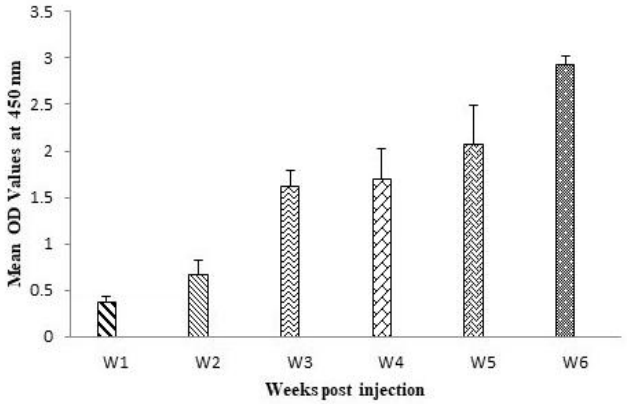

Fig. 2. Serum IgG titers in sheep immunized with purified camel immunoglobulin formulated with adjuvant measured by ELISA. The sera were collected prior to or at 7, 14, 21, 28, 35, and 42 days post-immunizations. Averages of duplicate samples were represented with standard deviations.

Discussion

Camels are susceptible to pathogenic agents. The availability of serodiagnostic tests help in diagnosing of infectious diseases in camels. These diagnostic immunoassays need anti-camel antibodies that are not available in the market and need to be imported. The current study is first attempt to develop new reagents (sheep anti-camel Ig polyclonal antibody) for immunological research and investigation. Polyclonal and monoclonal antibodies could be used in various types of bioassays in animals to detect specific antigens. If an antigen has various epitopes, polyclonal antibodies act better than monoclonal. Polyclonal antibodies connect to more sites of antigens resulting in better sensitivity.[10] IgG is the predominant immunoglobulin in humoral immunity and has the longest half-life of all immunoglobulin isotypes in serum.[11] Therefore, IgG plays critical role in protection against diseases, and can help in estimating and characterizing immunological responses.[12,13] To the best of our knowledge, in our country, this is the first report of using sheep for the production of anti-camel Ig polyclonal antibody. Sheep anti-camel Ig polyclonal antibody was successfully produced and antibody specifically reacted with camel serum Ig but had no cross-reactivity with Ig of horse and chickens. Previously, in Egypt, goat and rabbit anti-camel Ig polyclonal antibody were generated.[14] Polyclonal Abs with high affinity are useful tools in biomedical and biochemical research and they find application in the immune-assays for the detection and quantitation of camel IgG subclass antibodies levels. Antibody against camel IgG is suitable for conjugation with enzymes, radiolabels and fluorochromes.[6,15]

In conclusion, present study demonstrated the possibility of production of specific antibody against camel Ig in sheep indigenously. Newly produced indigenous polyclonal antibodies against camel Ig were cost effective antibody resource that will be used for immunoassay purposes in local camel population.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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