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PRODUCTION OF POLYCLONAL ANTIBODY AGAINST RAT IMMUNOGLOBULINS IN GOATS

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ABSTRACT

Objective: The goal of this project is taking a step towards self-efficiency with minimum expense. Its objective is producing an antibody in goats against rat immunoglobulins. The polyclonal antibody has various educational and diagnostic applications.

Methods: A fraction rich in immunoglubolins was saturated with ammonium sulphate to precipitate serum immunoglobulins. After several injections of rat immunoglobulins with adjuvant, in defined time periods, a goat was completely immunized. Then blood was taken from the animal for evaluation of goat anti-rat immunoglobulins using Ouchterlony and SRID tests. Antibody titration was done using the designed ELISA test

Results: In the Ouchterlony or double diffusion test, a clear precipitation line was seen and in the SRID test a clear precipitation ring was seen.

Discussion: The results of titration with designed ELISA tests and careful evaluation of antibody titer confirmed the results of double diffusion and SRID tests. The serum titer of immunized goats in this test was defined as 16000.

Conclusion: Production of high titer antibody in goats against rat immunoglobulins confirmed the results of Double Diffusion and SRID tests and so shows that this product is a cost-beneficial product.

Key Words: Polyclonal antibody, Rat immunoglobulins, Goat

INTRODUCTION

Antibodies are proteins that are produced in response to foreign proteins (antigen) by B-cells of the immune system. Polyclonal antibodies are produced in an immunized animal as a result of primed different clones of B-cells by various epitopes of antigen. From the quantitative point the amount of antibody is related to the amount of serum that is obtained from the immunized animal (1,2).

Polyclonal antibodies include immunoglobulins with different classes, subclasses, specificities, titers and affinities (3). Goat anti-rat immunoglobulins have various diagnostic and academic applications including: teaching double diffusion and single radial immuno diffusion (SRID) tests to students of medicine, pharmacy, and veterinary medicine. Also anti-rat Igs that are purified by affinity chromatography can be used for more purification of rat Igs. Furthermore, purified and conjugated goat anti-rat Igs can be used for designing ELISA kits and also for performing ELISA and Immunoblotting tests for purposes of diagnosing infectious diseases such as leishmaniasis(4).

Rats are very useful and common animals in immunological research. They have been used for a long time as models for study of different diseases specifically autoimmune diseases (5). Recently rats have been used as models of antimyeloperoxidase-associated proliferative glomerolonephritis (6). They are the selection of choice in the study of tissue transplantations. It is not surprising that the technology of inducing definite remaining of allogenic transplant with intrathymus injection of alloantigen was done for the first time on rats (7,8)

MATERIALS AND METHODS

In order to produce polyclonal antibody against rat Igs in a goat, different serum specimens from several rats were taken and were then mixed together. The pooled serum was diluted with phosphate buffer saline (PBS) pH=7.4 at a ratio of 1:1 for preparing rat immunoglobulin rich fractions. Saturated

ammonium sulfate was added to the diluted serum while it was being stirred. An immunoglobulin rich precipitation was formed at the bottom of the container with albumin remaining in supernatant.

In order to eliminate the proteins with low molecular weight from Igs, ammonium sulfate with the final concentration of 50% was used and was repeatedly centrifuged. Dialyzing the resultant precipitate against PBS, eliminated ammonium sulfate and an immunoglobulin rich fraction was achieved. The dialysis bag used for this purpose had a specific cutoff for Igs of 10,000-12,000 dalton (9).

The amount of Igs in this fraction was measured using spectrophotometer in 280 nm and was calculated as mg/ml. One(1) mg of resultant Ig was mixed with 1 ml of complete Freund's adjuvant (CFA) and was injected subcutaneously and intramuscularly in a goat. Next immunizations were performed in order to increase the immune response. The second and third dosages of antigen that were mixed with Incomplete Freund's Adjutants (IFA) were injected at intervals of three weeks, and after four weeks the fourth immunization was fulfilled without adjutants.

After the third and final immunization, blood samples were taken from the goat and antibodies produced were investigated by performing double diffusion and single radial immuno diffusion (SRID) tests. Dissolved L-agarose in barbital buffer with pH=8.2 was used for this purpose. At first, agarose was melted completely in more than 100°Ċ. To achieve a thickness of 1.8-2 mm on the slide, the temperature of melted agarose should be decreased to 50-60°Ċ. To perform the double diffusion (ouchterlony) test, three wells, one in the middle and the other two at the sides about 0.6-0.8 cm apart from the central well, were made in the gel. Antigen (rat Igs), immune goat serum, and PBS were poured in the central, right, and left wells respectively. To perform SRID, 300 µl of goat anti-rat Igs was poured evenly into 3ml of the gel and only 10 µl of antigen (undiluted and ½ diluted rat Igs) was added to the left and right side wells, respectively. PBS was added at the central well. Results were studied after 48 hours of incubation at humid chambers (10).

To evaluate the titer of polyclonal antibody, a designed ELISA test was applied. Rat Igs at the bottom of the wells on ELISA plates were coated as antigen and goat serum was poured in different dilution after non-specific sites had been blocked with Bovine Serum Albumin (BSA). Then anti-goat IgG conjugated with peroxidase (rabbit anti-goat IgG conjugate, sigma) was applied. Finally, a specific substrate for peroxidase, Ortho Phenylene Diamin (OPD), was added and the color alteration due to the enzyme and substrate reaction was read at 492nm (11).

RESULTS

In order to survey production of polyclonal antibody in goat and evaluating effectiveness of immunization, the ouchterlony test was performed. After staining the gel with coomassie brilliant blue, a precipitation line with relatively sharp density, between antigen and antibody wells, was observed (fig.1).

In SRID, the completely clear precipitation ring around antigen wells was also spread out indicating the production of anti-rat Igs in a goat and confirming the result of the ouchterlony test. However, no precipitation ring was observed around the PBS well (central well) (fig. 2).

DISCUSSION

Due to the soluble nature of antigen, adjuvant was used during injections. In order to further establish and gradually release antigen, complete adjuvant at the first injection and incomplete adjuvant at the booster immunizations were used. Adjuvant primes CMI responses and amplifies T-cell and B-cell related memory (12, 13).

To evaluate the production of polyclonal antibody following immunization, qualitative ouchterlony and semi quantitative SRID tests were performed that are considered as secondary immunological tests evaluating the antigen-antibody complex formation through precipitation. The diagnostic precision of these tests is 18-30 μg/ml. The reaction would never happen in the case of low concentration of antigen or antibody and precipitation line will not appear. Therefore, the appearance of this conspicuous precipitation line or ring indicates the production of antiglobulin. It is necessary to explain that, formation of sharp band between antigen and antibody wells in fig 1 is due to production of polyclonal antibody against rat IgG which forms the main Igs of rat. Sharp band associated with weak and thin bands indicate production of polyclonal antibody against either rat Igs or are due to cross reaction between IgG and other Igs, because the main target was producing polyclonal antibody against rat Igs and not purified rat IgG.

In fig 2, the diameter of the precipitation ring formed around the right side well is less than the one around left side well, because antigen (rat Igs) has been diluted at a ratio of 1:1 with PBS and it is obvious that radial diffusion of undiluted antigen at the left side well is bigger and therefore the diameter of the related ring is bigger, too. However, lack of a precipitation ring around the central well (PBS well), in comparison with considerable diameters of rings around the right and left side wells are due to production of polyclonal antibody against rat Igs and confirm results of double diffusion test. To evaluate polyclonal antibody titer, designed ELISA test which is a quantitative test, was applied. Because of directed evaluation of antigen-antibody complex, the test has a high degree of precision (3). Determining a titer of 16000 in this test indicates the high quality of the product. Thus, this antibody is highly economical and regarding the volume of 100 ml of serum taken from each goat at each sampling, considerable amount of anti-rat Igs can be obtained which meets a large portion of the requirements of the country in terms of research or educational programs.

In more cases such as electron microscopy, polyclonal antibody acts better than monoclonal antibody, because where detection of antigen with various epitopes is the target of study, polyclonal antibody can connect to the more connective sites and therefore sensitivity detection will be enhanced.

Connection of polyclonal antibodies to several epitopes of an antigen associated with easy and rapid use of them have been caused polyclonal antibodies act as expensive reagents in research (6). Sometimes specificity of polyclonal antibody against a specific antigen in comparing with monoclonal antibody is further. Because unwanted cross-reactions are removable in the case of polyclonal antibodies, while removing of them in the case of monoclonal antibody are not possible, because only have a specific connection site (5).

CONCLUSION

Production of polyclonal antibodies is completely cost beneficial, because about 100 ml of serum can be obtained from a goat at every sampling. After precipitation of serum with ammonium sulfate, a fraction rich in anti-rat Igs is achieved and can be used in many educational and research tests. Also purified anti-rat Igs with ion-exchange and affinity chromatography could have various applications including production of polyclonal and monoclonal antibody against purified antibody (purified anti-rat Igs), preparing of enzymatic and fluorescent conjugates, and finally designing ELISA and gel diffusion kits.

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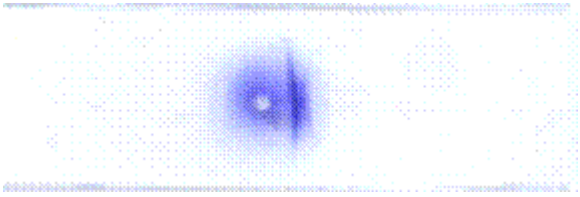

Fig 1: The result of Ouchterlony test indicates a sharp band between antigen and antibody wells.

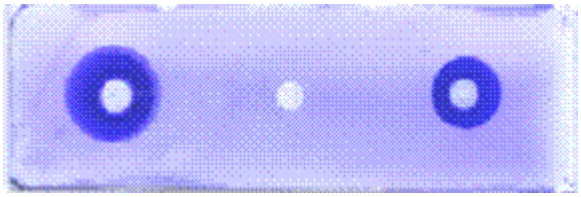

Fig. 2. Result of SRID test. The reaction between anti-rat Igs in the gel and rat Igs (diluted and undiluted) poured in the right and left side wells, respectively and PBS in the central well.

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