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PREPARATION OF ANTI-IDIOTYPE ANTIBODY AS DIAGNOSTIC KIT IN RABIES SEROLOGICAL TEST

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ABSTRACT

Serology remains the only way to monitor the effectiveness of vaccination of humans and animals against rabies. Many techniques for determining the level of rabies antibodies have been described, and all of the methods used rabies viruses as antigen. Indeed attenuated viruses can revert to a more virulent form, and inactivated virus may produce serious side effects. Anti-idiotype Ab can induce protective immune response against rabies virus, its means that anti-idiotype Ab can be used as surrogate antigen in serological test.The aim of this study is to prepare the anti-idiotype antibody (anti-idiotype Ab) as diagnostic kit in rabies serological test. Polyclonal anti-idiotype Ab were prepared in laying chickens and purified using affinity chromatography column for IgY. Rabbit anti dog immunoglobuline were prepared used *New Zeeland White* strain, and dog anti rabies serum were prepared from rabies immunized dog. For the preparation of the kit, it takes a few stages, i.e. the making of stocks A, stocks B and prototype diagnostic kit. Stocks A is a mixture of *S. aureus* Cowan I intact in solution of Tris Buffer HCl with rabbit anti dog serum in various comparisons (v/v). Stocks B is a mixture of rabies anti-idiotype Ab with rabies antibody (IgG anti rabies) are harvested from dog.Comparison of the obtained between whole *S. aureus* Cowan I and rabbit serum anti dog is 4~6:2~4. Optimization of stocks A and B based on the principle as follows: a merger of stocks A and B must not cause coagglutination. Formulation of aqueous stocks A and B will be a candidate when the diagnostic kits on the positive control produces coagglutination and negative controls do not produce coagglutination. The conclusions of this study is the anti-idiotype antibody can used and prepared as kit diagnostic with the principle of coagglutination by utilizing A protein of S. aureus.

Keyword: antibody, anti-idiotype antibodi, rabies, kit diagnostic, serological test

Introduction

Rabies is a viral disease of mammals and is transmitted primarily through bites. Detection and quantification of rabies antibodies is intended in the first place for checking the immunity to rabies or effectiveness of rabies vaccines. Detection and quantification of virus neutralisation rabies antibodies in the serum is based on inhibition of rabies infection in vivo in animals or in vitro in cell cultures (Ondrejková, 2002).

Serology remains the only way to monitor the effectiveness of vaccination of humans and animals against rabies. Many techniques for determining the level of rabies antibodies have been described, and all of the methods used rabies viruses as antigen. Indeed attenuated viruses can revert to a more virulent form, and inactivated virus may produce serious side effects.

The idiotype of an immunoglobulin molecule is formed by the total set of idiotopes, i.e., antigenic determinants on the variable region of an immunoglobulin molecule which are recognized by anti-idiotype antibodies.The specific part of the variable region that binds to the antigen is referred to as the paratope. Jerne (1974) originally proposed that a series of idiotypic-anti-idiotypic interactions regulate the immune response to an antigen. Interaction

between idiotypes of both antibody molecules and lymphocyte antigen receptors may form an idiotypic network in which a state of dynamic equilibrium exists.

Anti-idiotype antibody or anti-Id Ab refers to an antibody which identifies an epitope in the variable region of an idiotype antibody. A portion of such antibodies will identify an epitope that is the parotope of the idiotype antibody, thus presenting an "internal image" of the epitope detected by the idiotype antibody on the virus particle. So, anti-idiotype Ab can induce protective immune response against rabies virus and can be used as an alternative for rabies anti-idiotype vaccine(Paryati, 2009),or can be used as surrogate antigen in serological test.

Protein A is well known as one of the surface componentsof the cell wall of bacteria and is found in Staphylococcusaureus (Scriba et al. 2008). Protein A isa polypeptide with a molecular weight of 13-45 kDa, and bindscovalently with the cell wall of bacteria such as S. Aureus(Wibawan et al. 2009). Protein A has an important role in themechanism of infection, binding the crystallizable fragment(Fc) of mammalian IgG, but not Fc of IgY from chicken (Boyleet al. 1985). Protein A is able to bind the Fc of IgA and IgM(Langone et al. 1978). Protein A binds the Fc of IgG, suchthat the epitope in antigen binding fragment (Fab) is still freeand has capacity to bind specific antigens (Ribeiro and AraújoJr 2009).

The aim of this study is to prepare the anti-idiotype antibody (anti-idiotype Ab) as diagnostic kit in rabies serological test using field strains of *S. aureus* for coagglutination tests.

Materials and Methods

Polyclonal anti-idiotype (anti-Id) antibodies were prepared in laying chickens against anti rabies serum (ARS), called antibody 1 (Ab1). ARS was used as the antigen to immunize laying chickens. Anti-Ab_1 chicken immunoglobulins (IgY), the anti-idiotype antibodies against rabies (Ab_2) was isolated from chicken blood every week for ten weeks. Immunoglobulin Y was separated by means of ammonium sulfate precipitation, then dialyzed using PBS pH 8.0 for 24 hours at 2–8 ^oC and purified using affinity chromatography column for IgY.

In this research, rabbit anti dog immunoglobulin were prepared used *New Zeeland White* strain and dog anti rabies serum were prepared from rabies immunized dogs. Rabbits were vaccinated in the first week with 0.5 mL purified dog immunoglobulin (IgG) (1 mg mL-1, intra vena/iv), the vaccination was repeated in the second week and the third week with 0.5 mL purified IgG (3X, iv), respectively. One week after the last vaccination, the serum was collected and tested for the presence of specific antibody (IgG) against dog IgG using the agar-gel-precipitation test (AGPT) (Zhou et al. 1994)

Dogs were immunized with rabies vaccine subcutaneously (s.c), blood was taken 2 weeks after immunization for antibody titer tested. If the antibody titer is high enough, the dog blood was taken for the preparation of diagnostic kits. Blood stored for 30-60 minutes at 37^0C. After shaped clot (clotting), blood was stored at 4^0C for one night. Separation of serum performed by centrifugation 1500 rpm for 10 min at 4^0C.

Staphylococcus aureus with protein A present is used as carrier matrix coupled to the complex IgG-IgG-anti-idiotype Ab. The bacteria was preserved using formaldehyde 0.5% (v/v) for 60 min (Ansorg and Zarifoglu 1986). To avoid the self agglutination, box titration was done to find the appropriate composition of bacterial suspension, rabbit sera, IgG and anti-idiotype Ab, and this complex agglutinator is known as prototype for proposed diagnostic kits

For the preparation of the kit, it takes a few stages, i.e. the making of stocks A, stocks B and prototype diagnostic kit. Stocks A is a mixture of *S. aureus* Cowan I intact in solution of Tris Buffer HCl with rabbit anti dog serum in various comparisons (v/v). Each mixture was shaken until well mixed and then incubated at least 60 min at room temperature, then the solutions are stored in a sealed tube at a temperature of 4^0C.

Stocks B is a mixture of rabies anti-idiotype Ab with rabies antibody (IgG anti rabies) are harvested from dog. Serum was inactivated at 56^0C in a water bath for 30 minutes, then performed a serial dilution with a dilution factor of 2 between the stock mixture B with physiological saline. The mixture was shaken until homogen and then incubated for at least 60 minutes at room temperature and the solution was stored in a sealed tube at a temperature of 4^0C.

Optimization is done on the stocks A and B, using the following principles: 1). Merger between preparations A and B should not have coagglutination; 2). Formulation stock solution A and B will be a candidate diagnostic kits when the positive control produces coagglutination and the negative control did not produce coagglutination. Optimization of a mixture of A and B is done by taking into account: (1) the concentration of S. aureus inactivated, (2) anti-rabbit IgG serum titers of dogs, (3) anti-rabies titer (4) anti-idiotype Ab titers, (5) the incubation period, (6) the temperature of the mixture AB, (7) the temperature of the serum to be examined, (8) the speed and duration of centrifugation.

Results and Discussion

Two weeks after last immunization, chicken sera harvested from chicken immunized, and tested with AGPT showed the positive reaction signed by precipitation line among serum and ARS (Fig. 1). This indicated that chicken can produce antibodies that induced by ARS. The highest titer of Ab_2s was found at the third week after the last immunization. The antibodies Ab_1s made in response to the original antigen becomes itself an antigen and elicits the synthesis of a secondary antibodies, or Ab_2s. Ab_2s produced against the combining-site idiotope may carry an internal immage of the external antigen and are also known as internal-immage antidodies(Ban *et al*. 1994).

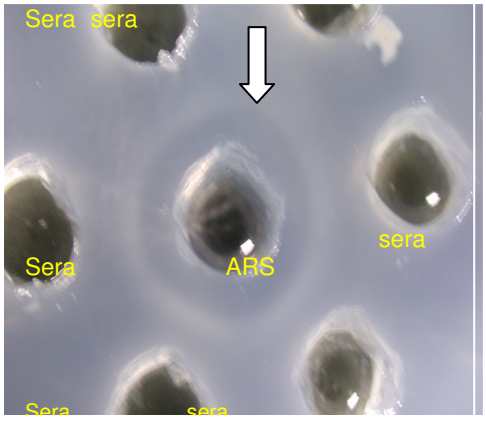

Fig. 1. Positive reaction with AGPT showed by precipitation line (arrow)

The highest titer of Ab_2s was found at the third week after the last immunization (Fig. 2). According to the Jerne hypothesis, the immune system responds to foreign substances as a regulatory networks composed of idiotypes (Ab_1s) and their anti-idiotypes (Ab_2s). The antibodies Ab_1s made in response to the original antigen becomes itself an antigen and elicits the synthesis of a secondary antibodies, or Ab_2s. This response can be divided into an antigen-noninhibitable group ($Ab_2\alpha$) and an antigen-inhitable group ($Ab_2\beta$). A third group, which is antigen inhitable because of steric hindrance with antigen binding site, is designated $Ab_2\gamma$.

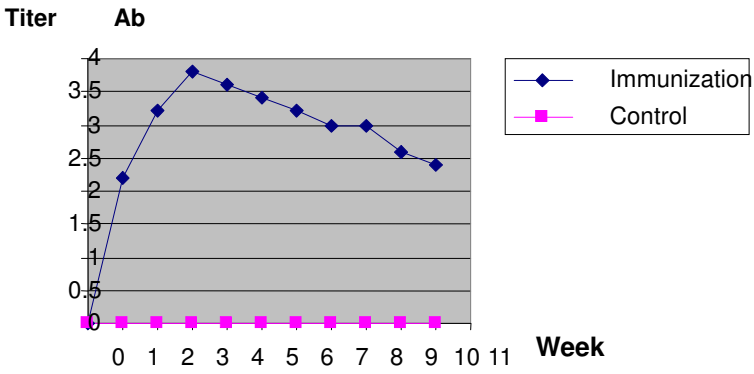

Fig. 2. Graphic of anti-idiotype antibody titer of chicken sera

Idiotypes are the some of idiotopes or serologically determined antigenic determinants unique to an antibody or group of antibodies. Ab2s produced against the combining-site idiotope may carry an internal immage of the external antigen and are also known as internal-immage antidodies. A true internal-immage molecule can induce immune-mediated responses similar to induced by original antigen, and this property has, in fact, been used to produce vaccines. Thus they may be significant structural mimicry between the complementarity-determining regions (CDRs) of internal immage Ab2s and this original antigen (Ban *et al.* 1994).

In addition to their presence as circulating molecules, immunoglobulins are found on the surface of B cells, and immunoglobulin-like structures comprise the T-cell receptor for antigen. All these molecules bear idiotypes. Thus, idiotype-antiidiotype interactions will involve antibody-cell and cell-cell interactions as well as antibody-antibody interactions. It is by involving cell regulation that the potential for suppression or enhancement of antibody production may occur. The precise mechanisms by which idiotype-antiidiotype interactions help regulate theimmune response are not known. Initial studies merely examined antibody-B-cell interactions, but recent advances in the understanding of T-cell receptor structure and function has focused attention on reports of induction of idiotype-specific 'suppressor' and cytotoxic T cells (Shoenfeld 2004; Nisonoff 1991).

Protein purification using affinity chromatography column for IgY than characterized using *Sodium Dodecyl Polyacrylamide Gel Electrophoresis* (SDS-PAGE), lead the proteins molecules (Ab2s) with 185,000; 95,000 and 49,000 dalton in weigth, and this result same with the weight of IgY, is about 180,000 dalton (Fig.3)

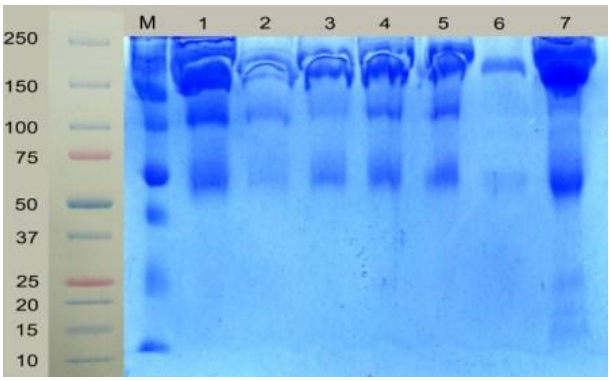

Fig. 3. Profile of protein bands were purified by affinity chromatography (column 6) showed the protein bands with molecular weights of 180 and 60 kDa compared with a marker (M).

Furthermore, the IgY anti-idiotype Ab (Ab_2s) was prepared as diagnostic kits. Stocks A is a mixture of whole *S. aureus* Cowan I intact in solution of Tris Buffer HCl with rabbit anti dog serum in various comparisons (v/v). Comparison of the obtained between whole *S. aureus* Cowan I and rabbit serum anti dog is 4~6:2~4. To avoid false positive reaction or to increase specificity, the normal rabbit IgG added to obtained the saturated solution. This done to prevent any bond with sample of serum negative (IgG in the serum examined) with A protein of*S. aureus* Cowan I that can cause coagglutination (Fig. 4).

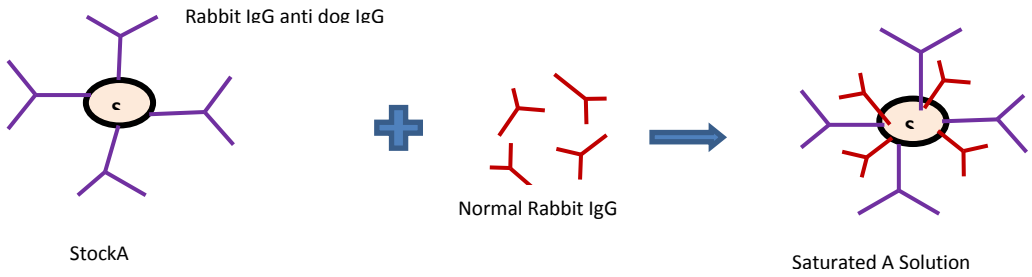

Fig. 4. Scheme of saturated A solution formula

Stocks B is a mixture of rabies anti-idiotype Ab with rabies antibody (IgG anti rabies) are harvested from dog. Optimization of stocks A and B based on the principle as follows: a merger of stocks A and B must not cause coagglutination. Formulation of aqueous stocks A and B will be a candidate when the diagnostic kits on the positive control produces coagglutination and negative controls do not produce coagglutination (Fig.5).

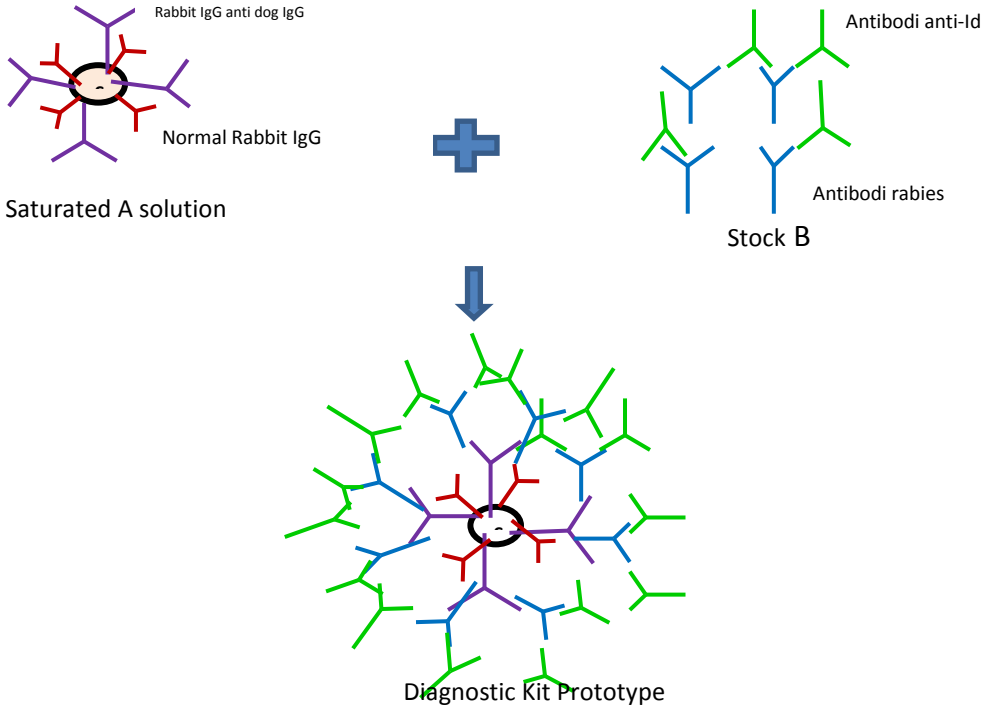

Fig. 5. Formulation of aqueous stocks A and B

This complex form is made and optimized using box titration to avoid the self agglutination reactions. This is the most crucial step in preparing the kits and the process is influenced by (i) the titre of IgG in rabbit sera to dog IgG, (ii) concentration of protein A of *S. aureus*, (iii) the titre of IgG anti rabies, (iv) the titre of anti-idiotype antibodies, (v) incubation period and (vi) the washing step.

Conclusions

The conclusions of this study is the anti-idiotype antibody can used and prepared as kit diagnostic with the principle of coagglutination by utilizing A protein of S. aureus. The prototype of the Diagnostic Kit was designed in complex form, using *S. aureus* containing protein A, rabbit sera containing IgG-anti dog IgG), purified IgG anti-rabies antibodies and anti-idiotype antibodies as surrogate antigen. This complex form is made and optimized using box titration to avoid the self agglutination reactions. This is the most crucial step in preparing the kits and the process is influenced by (i) the titre of IgG in rabbit sera to dog IgG, (ii) concentration of protein A of *S. aureus*, (iii) the titre of IgG anti rabies, (iv) the titre of anti-idiotype antibodies, (v) incubation period and (vi) the washing step.

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