

Antibodies titer of dogs immunized by anti-idiotypic vaccine detected by using enzyme linked immunosorbent assay

¹Sayu Putu Yuni Paryati, ²Retno D. Soejoedono, ¹Eka Noneng Nawangsih

¹Department of Microbiology, Medical Faculty, Jenderal Achmad Yani University, Jalan Terusan Jenderal Sudirman, Cimahi, ²Department of Immunology, Veterinary Faculty, Bogor Agricultural Institute, Bogor, Indonesia. Corresponding Author: yunisayu@yahoo.com

Abstract. Rabies control programs, including extensive vaccination with attenuated or inactivated vaccines. However, such vaccines are not without problems and can have detrimental effects. Indeed attenuated vaccines can revert to a more virulent form, and inactivated vaccines may produce serious side effects. These facts, have led to the creation of a new generation of vaccines: recombinant-DNA vaccines, synthetic peptide vaccines, and anti-idiotypic vaccines. The aim of this study is to study the result of anti-idiotypic immunization methods in dogs detected by using enzyme linked immunosorbent assay (ELISA). Anti-idiotypic antibodies against rabies (Ab₂) were isolated from chicken blood, separated by means of ammonium sulfate precipitation, then dialyzed using PBS pH 8.0 for 24 hours at 2 – 8 °C and purified using affinity chromatography column. Three groups of dogs were immunized, group I was immunized intramuscularly (i.m) with purified IgY, group II was immunized orally (p.o) with purified IgY and group III was immunized intramuscularly (i.m) with rabies viral vaccines. The antibody response (Ab₃) was detected using Agar Gel Precipitation Test (AGPT). The efficacy of Ab₃ was detected using ELISA. By ELISA, the result of immunization indicated that the level of Ab₃ titers of anti-idiotypic vaccine immunized dogs intramuscularly are more than 0.5 IU/ml (protective according to WHO standard), and significantly higher than orally immunization, but it significantly lower than Ab₃ titers of rabies viral vaccine immunized dogs. The conclusion of this study is intramuscularly immunization of anti-idiotypic antibodies can induce protective immune response against rabies virus, although its lower than antibodies titer of viral vaccine, it has a good prospect for vaccine development in controlling rabies.

Keywords: antibody, anti-idiotypic vaccine, rabies, ELISA

Introduction

Rabies is a zoonotic viral disease which infects domestic and wild animals. Once symptoms of the disease develop, rabies is fatal to both animals and humans. Humans typically contract the disease when bitten by infected domestic or wild animals. Rabies control programs, including extensive vaccination with attenuated or inactivated vaccines. However, such vaccines are not without problems and can have detrimental effects. Indeed attenuated vaccines can revert to a more virulent form, and inactivated vaccines may produce serious side effects. These facts, have led to the creation of a new generation of vaccines: recombinant-DNA vaccines, synthetic peptide vaccines, and anti-idiotypic vaccines.

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-idiotypic antibodies. The specific part of the variable region that binds to the antigen is referred to as the paratope. Jerne originally proposed that a series of idiotype-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. Administration of antigen disturbs this equilibrium and evokes an immune response. Antibody (Ab₁) will be produced in response to this external stimulus. Since antibody molecules bear immunogenic idiotopes and because of the diversity and completeness of the antibody repertoire, anti-idiotypic antibody (Ab₂) will be formed. Many reports have been published supporting this concept that idiotypes may function as targets of regulatory sign of idiotopes, i.e., antigenic determinants on the variable region of an immunoglobulin molecule which are recognized by anti-idiotypic antibodies (Ban *et al.* 1994).

Anti-idiotypic antibody can induce protective immune response against rabies virus and can be used as an alternative for rabies anti-idiotypic vaccine. Efficacy of Ab₃ was detected using enzyme linked immunosorbent assay (ELISA) indicated that the level of Ab₃ titer is protective according to WHO standard (more than 0.5 IU/ml) (Paryati, 2009). For the application of anti-idiotypic vaccine, its required to test of potency vaccine on animal laboratorium. The aim of this study to evaluate potency of rabies anti-idiotypic (Ab₂) for their immunogenicity and protection.

Materials and Methods

Polyclonal anti-idiotypic (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-Ab₁ chicken immunoglobulins (IgY), the anti-idiotypic antibodies against rabies (Ab₂) 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 °C and purified using affinity chromatography column for IgY.

Potency test were done on animal model. Three groups of dogs were immunized, group I was immunized intramuscularly (i.m) with purified IgY, group II was immunized orally (p.o) with purified IgY and group III was immunized intramuscularly (i.m) with rabies viral vaccines. Immunization were done at 0, 7th and 14th day (WHO, 2002). The antibody response (Ab₃) was detected before immunization and then at 2, 4, after , 12 and 16 week pasca immunization using Agar Gel Precipitation Test (AGPT). The efficacy of Ab₃ was detected using enzyme linked immunosorbent assay (ELISA).

Results and Discussion

Anti-idiotypic Immunoglobulin

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 Ab₂s was found at the third week after the last immunization (Fig. 3). According to the Jerne hypothesis, the immune system responds to foreign substances as a regulatory networks composed of idiotypes (Ab₁s) and their anti-idiotypes (Ab₂s). The antibodies Ab₁s made in response to the original antigen becomes itself an antigen and elicits the synthesis of a secondary antibodies, or Ab₂s. This response can be divided into an antigen-noninhibitable group (Ab_{2a}) and an antigen-inhibitable group (Ab_{2β}). A third group, which is antigen inhibitable because of steric hindrance with antigen binding site, is designated Ab_{2γ}.

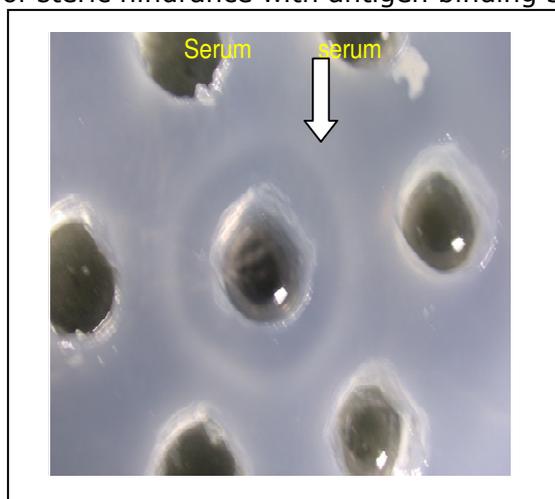


Fig. 1. Positive reaction with AGPT showed by precipitation line (arrow)

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 image of the external antigen and are also known as internal-image antibodies. A true internal-image 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 image 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 the immune 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).

Purification of Immunoglobulin

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

is about 180,000 dalton. The antibody response (Ab₃s) was detected using Agar Gel Precipitation Test (AGPT) showed specific reaction with Ab₂ as well as rabies virus. Interestingly, Ab₂ and rabies virus express partial identity reaction. This lead the insight, that not all parts of Ab₂s are internal image of rabies virus. In 1978, Kohler proposed a new function of antibodies in the regulation of immune responses. Antibodies have specific variable sequence determinants which are detected by anti-idiotypic antisera. Anti-idiotypic antibodies can suppress the production of the complementary idiotype antibody and are, therefore, specific anti-receptor antibodies. Experiments in inbred mice have shown that mutual and reciprocal functional interactions of idiotype and anti-idiotype can occur. These findings give evidence for regulatory mechanisms in the expressions of immune clones by complementary idiotypes. An idiotype actually consists of multiple antigenic determinants, each of which is an idiotope. The antigenic determinants or idiotopes can reside in the heavy chain component of the V region, in its light chain component, or they may consist of a surface made up of parts of both chains. The idiotype of an antibody is, therefore, a way to describe by serological means the variable region of an antibody molecule. Originally, idiotypes were defined by heterologous antisera made, for example, by immunizing a rabbit with a monoclonal human myeloma protein. After extensive absorption with normal human immunoglobulins, such antisera were found to bind only to the V region of the immunizing myeloma immunoglobulin. Thus, idiotypes were originally thought to be unique markers of individual antibodies (hence their name). However, it is now known that different antibodies can share the same, or similar, idiotype. Even antibodies with different antigen-binding specificities can share the same idiotype if they use the same heavy or light chain in forming their V regions. Such antibodies constitute parallel sets. Thus, idiotypes can be: private (confined to a particular immunoglobulin molecule), public (shared by different antibodies), or cross-reactive (different idiotypes containing similar antigenic structures) (Migliorini and Schwartz 1988).

Potency Test

The efficacy of Ab₃s detected using enzyme linked immunosorbent assay (ELISA) indicated that the level of Ab₃s titer is protective according to WHO standard (more than 0.5 IU/ml). Anti-idiotype antibody can induce protective immune response against rabies virus and can be used as an alternative for rabies anti-idiotype vaccine (Paryati *et al.* 2006). These findings indicate that Ab₂ induced the formation of antigen-specific Ab₃, probably because it bears the internal image of the rabies virus. This Ab₂ may therefore have potential for modulating the immune response (Herlyn 1986).

Table 1. Means of rabies antibodies titer

Group	Rabies antibody titer of dog pasca immunization (IU/ml)					
	Pre-immunization	week				
		2	4	8	12	16
p.o. (IgY)	0,05	0,11	0,10	0,22	0,35	0,26
S.c. (IgY)	0,15	0,20	0,30	0,52 *	0,59 *	0,52 *
S.c (rab)	0,09	0,47	0,96 *	1,03 *	0,82 *	0,57 *

Remarks:

- p.o (IgY) : immunization with crude immunoglobulin per oral
- s.c (IgY) : immunization with purified immunoglobulin (IgY) subcutaneous
- S.c (rab) : immunization with commercial rabies vaccine subcutaneous
- Mark (*) : Protective titer antibody of rabies (> 0,5 IU/ml)

Statistical analysis (Table 1) of Ab₃s titer indicated that the Ab₃s titer of anti-idiotypic immunization per oral (p.o) were not yielded protective titers. Its might be that immunoglobuline Y were damaged by digestive enzymes. Antibodies titer of anti-idiotypic vaccine immunization (s.c) on the eighth week until 16th week post immunization were protective (yielded > 0,5 IU/ml of the antibodies titer) but very significantly (P<0,01) lower than the level antibodies induced by viral rabies vaccine on the forth until 16th week post immunization. This indicated that anti-idiotypic antibodies could not induce the same level of antibodies induced by viral vaccine. But theoretically, anti-Id antibodies may provide advantages as vaccines over conventional or even some recombinant DNA based vaccines, including: (1) the lack of risks

associated with live pathogens; (2) relatively inexpensive preparation compared to propagation of virus; (3) the fact that anti-Id antibodies mimic the native 3-dimensional conformation of the antigen and, therefore, may stimulate a better immune response than inactivated or subunit vaccines which may have been altered in antigenicity; and (4) the fact that some anti-Id antibodies have the potential to stimulate both humoral and cellular immunity.

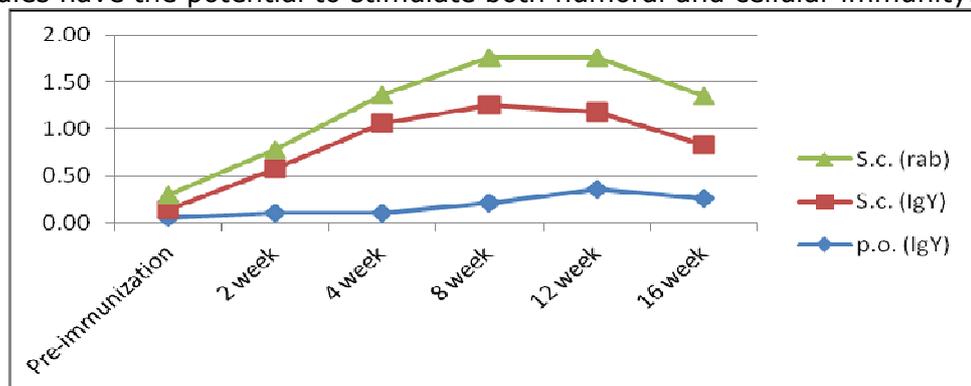


Fig. 2. Graphic of antibodies titer harvested from immunized dog

Conversion of OD values to International Unit (IU) indicated that antibodies titer of sera harvested from dogs immunized with anti-idiotypic antibodies were higher than 0.5 IU at the fourth week until the 16th week (Fig 2). This means that anti-idiotypic antibodies can induce protective antibodies against rabies (according to WHO standard) (OIE 2004). This titer was decreased after 16 weeks p.i.

Conclusions

The conclusions of this study are (1) the anti-idiotypic antibody can induce protective immune response against rabies virus and can be used as an alternative for rabies anti-idiotypic vaccine, (2) the efficacy of Ab₃s was detected using enzyme linked immunosorbent assay (ELISA) and indicated that the level of Ab₃ titer is protective according to WHO standard (more than 0.5 IU/ml), (3) statistical analysis of Ab₃s titer indicated that the Ab₃s titer of anti-idiotypic immunization were protective started from 4th until 16th pasca immunization, significantly higher on the 8th until 16th week post immunization compared with control serum (P<0.05) but significantly (P<0,01) lower than the level antibodies induced by viral rabies vaccine on the four until 16th weeks post immunization, (4) this titer was decreased after 16th weeks post immunization

References

- Ban N, Escobar C, Garcia R, Hasel K, Day J, Greenwood A, McPherson A. 1994. Crystal Structure of Idiotype-Anti-Idiotype Fab Complex. *Proc. Natl. Acad. Sci. USA*. 91:1604-1608.
- Guancheng L, Jinyue H, Guohua Z, Jiangaio Z, Qubing S. 2001. Monoclonal Anti-idiotypic Antibody Bearing The Internal Image of Nasopharyngeal Carcinoma Associated Antigen. *Chin. Med. J.* 114(9):962-966.
- Herlyn D, AH Ross, and H Koprowski (1986). Anti-idiotypic antibodies bear the internal image of a human tumor antigen. *Science*. 232(4746):100 – 102.
- Jerne, N. K. (1974) *Ann. Immunol. Inst. Pasteur* 125C, 373-389.
- Migliorini P, R S Schwartz. (1988). Anti-idiotypic antibodies in autoimmune diseases. *Clin Exp Rheumatol*. 6(2);145-50.
- Nisonoff A. 1991. Idiotypes : Concepts and Applications. *J. Immunol.* 147(8):2429-2438.
- OIE. 2004. Rabies. In: Manual Diagnostic Tests and Vaccines for Terrestrial Animals. 5th ed. OIE World Organisation for Animal Health. Francis.
- Paryati SPY, Wibawan IWT, Soejoedono RD, Pasaribu FH. (2006). Chicken immunoglobulin as the anti-idiotypic antibody against rabies. *J. Vet.* 7(3) : 92-103.
- Shoenfeld Y. 2004. The Idiotypic Network in Autoimmunity : Antibodies that Bind Antibodies. *Nat. Med.* 10:17-18.
- Sollazzo M, D Castiglia, R Billetta, A Tramontano and M Zanetti (1990). *Structural definition by antibody engineering of an idiotype determinant*. Protein Engineering, 3:531-539, Oxford University Press
- WHO. 2002. Rabies vaccines. In : Immunization, Vaccines and Biologicals. *Weekly Epidemiological Record*, 77:109-120. <http://www.who.int/wer/pdf/2002/wer7714.pdf>. [22-06-2004].
- WHO. 2003. Discussion on WHO Requirements for Rabies Vaccine for Human Use : Potency Assay. Report. World Health Organization. Geneva.