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Production of Polyclonal Rabbit IgG against Dog IgG: Orientation for Applications in Immunological Diagnosis of Dog Diseases

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Abstract
Dogs are one of the most popular pets in many parts of the world. The need to diagnose dog diseases using immunological assays is constantly increasing. This study aims to produce anti-dog IgG antibodies from rabbits for use in immunological diagnosis in dogs. To achieve this, the dog's refined IgG is used as an antigen to cause an immune response in rabbits. Specific rabbit IgG is then purified from serum by precipitation method with 45% saturated amonium sulfate salt, protein G affinity chromatography and dog IgG affinity chromatography. The purity of rabbit IgG reached 95.3% based on SDS-PAGE analysis. The specific activity of purified IgG is confirmed by dot blot assay. These results suggest that rabbit anti-dog IgG antibodies produced in this study can be applied to immunological diagnosis of dog diseases.

Keywords: Affinity chromatography, Dog IgG, Immunological diagnosis, Protein precipitation, Rabbit IgG.

INTRODUCTION

Immunoglobin G antibodies are biological molecules that plays an important role in the animal immune system. They are produced by B cells after animals are infected with antigens from microorganisms or other foreign protein molecules. In canine, there are four IgG subclases which engage distinct functions in the immune system via complement-dependent cytotoxicity or antibody-dependent cell-mediated cytotoxicity [1]. The production of antibodies against dog IgG (dIgG) is necessary for development of diagnostic tools to detect many immunological imprints of infectious diseases [2, 3]. Experimental animals for antibody production are diverse, including rabbits, goats, mice and other animals [4]. Among them, rabbits are considered a good choice, suitable for laboratory conditions because they are moderate in size, gentle and easy to take care of. Additionally, they have large amount of serum which is easily upscaled to produce antibodies for applications in many biological techniques.

There are many different approaches to purify IgG from rabbits (rIgG) including protein precipitating with saturated ammonium sulfate solution, ion exchange chromatography, affinity chromatography (protein A, protein G, targeted antigens, metals, lectin) [5-7]. In this study, rabbits were used to produce anti-dog IgG antibodies. The purification method of specific rIgG consists of protein precipitate using saturated ammonium sulfate, affinity chromatography with protein G and dIgG. The purified rIgG can be used for diagnosis by immunological techniques in dog diseases.

MATERIALS AND METHODS

Experimental animals

Two rabbits (2 months old, 2 kg weight) were purchased from the Pasteur Institute in Ho Chi Minh City and were raised in our animal house in Faculty of Biotechnology, Nguyen Tat Thanh University.

Rabbit immunization

The dIgG (>95% pure) was used to induce an immune response in rabbits by intradermal injection according to previous published work [5] but with modification. The regimen of immunization is shown in Table 1. Rabbit back skin was intradermal injected with dIgG prepared in complete Freund's adjuvant (CFA) for the primary injection and in incomplete Freund's adjuvant (IFA) for the boosters. The blood was taken from the ear vein, except for the last time the blood was drained from the heart.

Table 1. Schedule for rabbit immunization with dIgG.

Protocol day	Procedure	Description
Day 0	Control serum collection	Pre-immune bleed (5 ml per rabbit)
Day 1	Primary injection	Immunize with 0.25 mg dIgG in CFA
Day 14	1st booster	Bleed (5 ml per rabbit) Immunize with 0.1 mg dIgG in IFA
Day 28	2st booster	Immunize with 0.1 mg dIgG in IFA
Day 42	Serum collection	Bleed (25 ml per rabbit)
Day 56	3st booster	Immunize with 0.1 mg dIgG in IFA
Day 70	Serum collection	Bleed (25 ml per rabbit)
Day 84	4st booster	Immunize with 0.1 mg dIgG in IFA
Day 98	Serum collection	Bleed (50 ml per rabbit)

Ouchterlony assay

The assay was used to check for specific antibody production in the rabbits after each times of dIgG injection [8]. 1.5% agarose and 1.5 mm thickness gel was prepared. Six wells (2 mm diameter) were created on the gel in hexagonal form with a length of 1 cm each and another 1 well was created in the central position. The 6 peripheral wells were loaded with 10 µl of rabbit diluted (by a factor of 2) or undiluted serum. The central well was loaded with 10 µl