Research Article

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DETERMINATION OF ANTIGEN-SPECIFIC ANTIBODY LEVELS IN FISH SERUM BY ELISA

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Abstract: Indian major carp, *Labeo rohita* was immunized with bovine serum albumin (BSA) and serum was collected on days 7, 14, 21 and 28 after immunization. The BSA-specific antibody levels were determined by enzyme linked immunosorbant assay (ELISA), without raising anti- fish sp. antibodies.

Keywords: *Labeo rohita*, Antigen-specific antibody response, Enzyme Linked Immuno Sorbant Assay (ELISA)

INTRODUCTION

Determination of antigen-specific antibody response in serum of any animal requires anti-species antibody. Anti-species antibodies were commercially available for human, mouse, rat, rabbit, horse, donkey, goat, sheep, pig, guinea pig, cow, dog, cat, monkey and chicken. Determination of antigen-specific antibody response in fish serum was usually performed by either direct hemagglutination or passive hemagglutination assays. The lack of anti-species-fish-IgM antibodies has forced the researchers to depend on the above agglutination methods. Some laboratories have prepared anti-fish Ig antibodies by isolating antibodies from the fish serum and injecting into a host, these are not commercially available but are limited to that laboratory only. Monoclonal antibodies to rainbow trout IgM (*Oncorhynchus mykiss*) were prepared and characterized for use in immunoassays (1). Serum immunoglobulins were purified and monoclonal antibodies against immunoglobulin heavy chain of common carp (*Cyprinus carpio*) was produced (2). Serum immunoglobulins of rohu (*Labeo rohita*) were purified by affinity chromatography using bovine serum albumin as capture ligand. The purified rohu Ig was used for the production of two anti-rohu Ig monoclonal antibodies (3). Though the monoclonal antibodies were prepared, these would lightly cross react with the other species of the family but not with other family species. There are several species of fishes and if one raised anti- species-fish-Ig antibodies, this antiserum will not be useful for determining the antibody levels of other species of fishes. In our laboratory we have performed ELISA by double antibody technique to determine the antigen-specific antibody levels in fish serum. Using this method one can determine antigen-specific antibody levels in any species of fishes.

MATERIALS AND METHODS

Experimental conditions:

Labeo rohita, rohu with average weight of 200±37g were obtained from the fish farm and were divided into different groups and acclimatized for 2 weeks before the start of the experiment. Fish were fed with artificial diet prepared in our laboratory. Temperature was ranged between 28-34°C, and DO levels were maintained above 5mg/l with the help of aerators throughout the experiment.

Immunization and sampling:

After acclimatization, all fish were anaesthetized with MS-222 and immunized intraperitoneally, with 10 mg of bovine serum albumin per fish. Sampling was done on weekly intervals, ie. On days 7, 14, 21 and 28 after immunization. Four fish were sampled at each time. Blood was collected by cutting the tail, and allowed to clot at room temperature and serum obtained by centrifugation was used in the experiment.

Enzyme linked immunosorbant assay (ELISA):

96 well flat bottomed ELISA plates Microlon 600 were purchased from greiner bio-one. Disodium hydrogen phosphate, sodium dihydrogen phosphate and sulfuric acid were purchased from Merck. Rabbit anti-BSA antibodies purchased from ICN Biomedicals Inc., USA and goat anti-rabbit Ig-antibodies conjugated to HRP purchased from Bangalore Genei. Tween-20 and hydrogen peroxide purchased from Qualigens. Skimmed milk powder and BSA purchased from SRL. o-phenylenediamine dihydrochloride from Sigma, USA.

An antigen-specific antibody level in fish (*L. rohita*) was determined by ELISA (Fig.1) as described hereunder.

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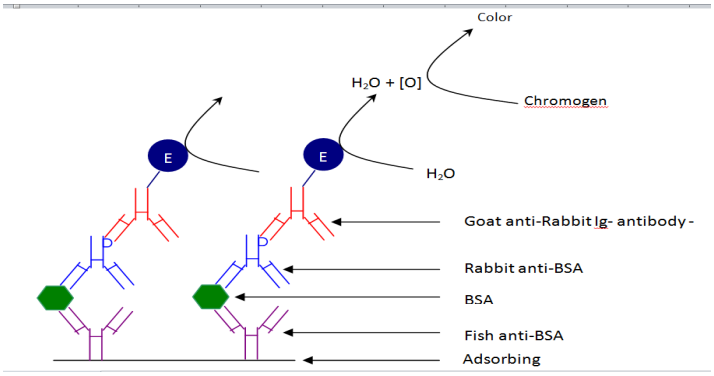

Fig.1: Schematic diagram for determination of antigen-specific antibody levels in fish by ELISA

Coating:

The wells of the microtiter plate will be coated with 100 µl of fish serum in serial dilutions in phosphate buffered saline pH 7.4. The plates were incubated for 12 hours at 4°C. After incubation the wells were washed three times with PBS containing 0.05% tween-20.

Blocking:

The free binding sites of the wells will be blocked by adding 5% skimmed milk powder diluted in PBS, 300 µl of per well. The plates were incubated for 12 hours at 4°C. After incubation, the wells were washed three times with PBS-tween.

Antigen:

100 µl of bovine serum albumin (BSA) dissolved in PBS were added to each well (100ng BSA/well). The plates were incubated for 2 hours at 4°C. After incubation, the wells were washed three times with BPS-tween.

1st antibody:

Rabbit anti-BSA serum (ICN Biomedicals, USA) diluted 1:500 in PBS was added, 100µl to each well. Plates were incubated for 1 hour at 37°C. After incubation, the wells were washed three times with PBS-tween.

Conjugate:

Goat anti-Rabbit-Ig antibodies conjugated to horseradish peroxidase (GENEI, India) was diluted 1:1000 in PBS and added to all wells, 100 µl per well. The plates were incubated for 1 hour at 37°C. After incubation, the wells were washed three times with PBS-tween.

Substrate:

13mg of o-phenylenediamine dihydrochloride (Sigma, USA) was diluted in 10ml of citrate-phosphate buffer (pH 5.0). 10µl of Hydrogen peroxide was added to the above solution before use. After washing the wells, the above substrate solution was added all wells, 100µl per each well, and incubated for 15 minutes. After developing the color, the reaction was terminated by adding 4N sulfuric acid, 50µl per each well. The optical density was measured at 490nm in an automatic microplate reader.

Determination of Antibody titer:

The highest dilution of the serum that gave the OD > 0.1 was taken as the specific-antibody titer.

RESULTS AND DISCUSSION

The results obtained were shown in Fig. 2. Here in this experiment it was observed that the specific antibody level was peaked in day-7 and then gradually declined towards day-28. The highest antibody titer was $1024x10^3$ (found on day-7), and lowest antibody titer was $8x10^3$ (found on day-28). This method of specific-antibody titer determination may be useful for species for which anti-species antibodies are not available. This method has been standardized and been used in our laboratory and few results using this method was published in standard journals (4).

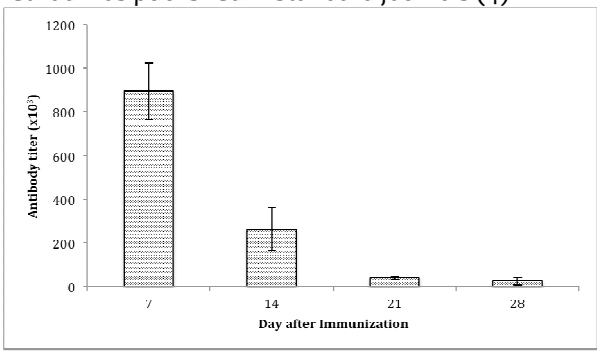

Fig.2. Antigen-specific antibody level in the serum of *L. rohita* was determined by ELISA, after immunizing the fish with BSA.

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