EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 4 Number 3 (March-2016) pp. 114-119

Journal home page: http://www.ijcrar.com

doi: http://dx.doi.org/10.20546/ijcrar.2016.403.013

Cell Mediated Immune Response in a Fresh Water Fish, *Catla catla* against a Bacterial Antigen

A. Thangaraj[1], G. Rekha[2] and P. Dhasarathan[3*]

[1]Department of Plant Biology & Plant Biotechnology, Dr.Ambedkar Govt.Arts College, Vyasarpadi, Chennai-39, India
[2]Department of Biotechnology, St.Peter's University, Avadi, Chennai – 600 054, India
[3]Department of Biotechnology, Prathyusha Engineering College, Tiruvallur – 602025, iNDIA
Corresponding author

KEYWORDS

Catla catla,
Bacterial antigen.

A B S T R A C T

In this investigation found a bacterial pathogen, *Pseudomonas aeruginosa* strain, two different of antigens such as whole cell antigen and nucleotide antigens were prepared and injected into the experimental fish(*Catla catla*) groups and control for the study of cell mediated immune response and cell mediated immune response. Cell mediated response analysed by T cell counts as whole bacterial antigen in fish showed the increased the productions of T cells in primary (56±1) and secondary immune response (58±2)compare with nucleotide antigen exposes is low. DTH response was showed inflammation at 3rd week of secondary response but postive reaction was showed in primary and secondary response. Lympocyte migration was showed in both types of strains fishes exposed inhibition of pathogens. The enhancement of this type of immune responses confirms the potential of chosen antigen to be used as a vaccine.

Introduction

The immune system is known to be involved in the etiology as well as pathogenic mechanism by many diseases. Immunology is the probably one of the most rapidly developing areas of biomedical research and has great promises with regard to the prevention and treatment of a wide range of disorder. Fish are the first group of vertebrate animals with both innate and adaptive immune responses and are essential for proper understanding of the immune system and its evolution. The fish adaptive immune responses are less effective than in mammals because they are pokilotherms and completely dependent on their environment temperature. Overall the mechanism and molecules involved in the immune responses are quite well conserved during the immune system evolution. Aquaculture represents one of the fastest growing food producing sectors. The uses of natural immunostimulants are in fish culture for the prevention of diseases in a promising new development in vaccine (Anderson, 1992; Ruscetti *et al.,* 1993; Tatiya *et al.,* 2007).

The immune system is composed of immune cells and proteins serve to defend the body against harmful bacteria, viruses, fungi and other foreign invaders. Activation of the immune system causes inflammation within the tissues where the activation occurs (Nawale Roshan and Poojari Savitri, 2013; Ghasis *et al.*, 2013).

T cells and B cells Which mediate cellular and serologic or humoral immunity respectively these are present in the circulating blood and peripheral tissues. The recognition of the antigen by T cells leads to proliferation of these cells, infiltration of immune cells at the site of action and cellular immunity. These reactions may be manifestated as delayed type hypersensitivity, tissue graft rejection (Gebel *et al.*, 1997; Goldsby *et al.*, 2003). B cells play an important role in the humoral immunity, together with T cells, they make up the third line of defense differentiating into specialized antibody producing plasma cells and memory cells after activation (Talaro, 2001; Sujatha and Dhasarathan, 2013).

Methodology

Experimental animal *Catla catla* collected from government fisheries pond and transported to the laboratory for accumulation. After accumulation animal were divided into three groups. Two groups of fishes were immunized with 1 ml of two different antigens (whole cell antigen, nucleotide antigen) of *pseudomonas aeroginosa* through intraperitoneal injection using 1 ml glass syringe with 24 gauge needle. The fish was held with lateral facing the investigator. The needle was inserted about half a centimeter just above the peritoneal cavity (which can be confirmed by the free movement of the free end). A group of fishes used as controls had received the same amount of saline.

Blood collected from immunized and normal fish was kept at room temperature for 15 minutes. The clot was freed from the wall of the micro centrifuge tube for efficient retraction and kept overnight at 4°C. The serum was separated by spinning down the clot at 3000 rpm for 15-20 minutes and collected in sterilized vials. The serum was stored in the freezer at −20°C until use. In the present study, cell mediated immune response was analyzed by DTH, lymphocyte migration and T cells rosette assay techniques were carried out.

T cell Erythrocyte Assay: Blood samples were collected from test antigen treated and control fishes in heparin pretreated vials. T-cell counts in the blood samples were carried out after isolation of lymphocyte from blood plasma. From the plasma lymphocytes were separated using ficol and centrifuged the lymphocyte layer alone. Lymphocytes were resuspended and loaded into activated nylon wool column. Then the column was held vertically above an eppendorf tube and now hot saline (about 60°C) was slowly dripped into the column. The hot saline passing through the column was collected in the eppendorf tube, contains T lymphocytes: 0.2ml of saline containing T Lymphocytes (from the eppendorf tube containing T cell) was taken in a separate eppendorf tube and 0.2 mL of 1% SRBC was added and then the mixture was centrifuged for 12 minutes at 1600 rpm. After centrifugation these samples were incubated in an ice box or in refrigerator at 4°C for 5 minutes. After cold incubation, the pellet in the eppendorf tube was resuspended by gentle flushing with Pasteur pipette. Then a drop of it was taken in a clean dry slide. Observed under the microscope (20x / 40x) and enumerated T cells for rosettes. Number

of rosettes formed in hemocytometer was observed per hundred lymphocytes observed and tabulated.

Delayed Type Hypersensitivity: Delayed type hypersensitivity was studied in dissolving following method of Tamang *et al*., (1988). The experimental fishes (control and 7 days antigen exposed fishes) were sensitized by a single subcutaneous application of 0.5 mL DNCB (10 mg/mL). A positive response is conventionally assessed as one giving ≥ in durations. Response can be graded as 3 – 4 mm = +; 5 – 8 mm = ++; 9 – 11 = +++; 12 mm or more = ++++. Fishes were sensitized by subcutaneous injection in the intranasal region with 0.5 mL of Freunds adjuvant containing 500 mg of antigen and boosted at 6th and 8th day by an intradermal injection to sterile phosphate buffer with a vernier caliper prior to challenge, i.e. 0th, 2nd, 4th, 6th and 12th hour post challenge, each with three readings. The increase in mean skin thickness (MST) of fishes was obtained after deducting the skin thickness of the same oil before challenge. Overall MST was obtained by taking the mean of individual fishes with a group.

Lymphocyte Migration Inhibition Test: Blood is collected from antigen treated and control fishes using a heparin pretreated vials. 5 – 10 mL of the blood was collected and it was introduced into sterile conical flask / beaker containing (4 – 5) sterile glass beads. It was then continuously swirled until no sounds heard from the vessel. This indicates that all fibrins have adhered to the beads. This blood was considered as defibrinated blood and diluted with equal volume of physiological saline. 3 mL of the lymphoprep solution was taken in a centrifuge tube using Pasteur pipette. Care was taken so that FICON layer of the lymphoprep solution present in the centrifuge tube was then centrifuged at 1600 rpm for 20 min. The interphase (containing lymphocytes) was removed using pipette. The cells were washed with 1 mL saline and excess FICON was removed. The cells after washing 3 times in Hangs balanced salt (HBSS) containing Heparin (5 mL) are suspended in Eagles minimum essential medium with 10% bovine serum. The viability of the cells was checked by tryphanblue dye exclusion method and the concentrations have to be adjusted to 1 x 107 cells / mL. The cells are packed in capillary tubes and fixed in Petridish to which added Eagles medium containing specific antigen then incubated overnight for migration.

Results and Discussion

The two different antigens prepared from *Pseudomonas aeroginosa* strains pathogen such as whole cell bacterial antigen and nucleotide antigen were administered in fish comparative study to character analysis of *Catla catla* fish exposed to different types of pathogenic antigen. The weight of the animal was weighed exposed to different types of antigen such as whole cell bacterial antigen and nucleotide antigen. The nucleotide antigen exposed fishes are abnormal movement and low adultation days, in which the whole cell bacterial antigen exposed fish also abnormal movement and moderate adultation days (Table 1).

The percentage of T cell counts in primary and secondary immune response against in whole cell bacterial antigen and nucleotide antigen at different time intervals (Table 2). The value of T cell productions at different time intervals in whole cell bacterial antigen showed the high value of immune response in primary and secondary immune response. Nucleotide antigen exposed fish has the

value of T cells are low immune response in first 3 weeks of primary response, but the T cell value is increased at end of third weeks in secondary immune response. Kougt *et al.* (1995) reported that good indications of cell mediated immunity plays an important role in controlling and destroying the intracellular pathogen. Thus the changes in cell mediated immunity and lymphocyte cell percentage are due to whole cell bacterial antigen and nucleotide antigen evident as immunostimulant activity in fish. The effects of pathogen improving the lymphocytes percentage, which leads to produce more T cells, undergo maturation in the thymus gland and play a major role in cell mediated immunity.

Alamelu Raja (2004) reported that CD4+ also role in controlling the microbial infection, CD8+ cells are capable of secreting of cytokines such as IFN-alpha and CD4+ role in regulating the balance TH1 and Th2 cells in infections. IFN-gamma is playing a role in direct modulation of heamatopoiesis during bacterial infection. The results showed that the augmentation of cell mediated immune response against the whole cell bacterial antigen and nucleotide antigen compared with control as evident that T cells are vital component in cell affected mediated immune response get stimulated by different pathogenic strains to improving the immune responses of primary and secondary response.

In the present study, DTH reaction was measured in primary and secondary immune response against different pathogenic strains at different time intervals (Table 3). The delayed type hypersensitivity was showed in negative results to exposure of whole cell bacterial antigen in primary response in first 3 weeks, in the condition of secondary immune response in last 3rd weeks showed the positive results of inflammation occur. Nucleotide antigen exposed fishes are positive inflammation seen in both primary and secondary immune response. A positive results showed that reaction indicates that individual has a population of sensitized Th1 cells to produce cytokines to induce the localized inflammation reaction specific for the test antigen, DTH response diagnosed based on the development of a red, slightly swollen, firm lesion at the site of injection between 48 hours to 72 hours.80%-90%n of these cells are macrophages (Goldsby *et al*., 2003). Tatiya *et al*. (2007) suggested increase in inflammation showed that antigenic challenge that appears depression of cell mediated immunity by thymus independent antigen. The reduce development of inflammation reaction in fish exposure that to antigen suggest a possible impairment in the immunocapacity of fish.

These results clearly established that nucleotide antigens are effective population of cytokines with Th1 cells to localized the inflammation to mediated the cell mediate immune response. But the whole cell bacterial antigen exposed fishes showed the negative reaction of inflammation indicates that impairment of cell mediated immune response. In the present showed that lymphocyte migration in primary and secondary immune response against whole cell bacterial antigen and nucleotide antigen at different time intervals (table 4). The results showed that whole cell bacterial antigen exposed in fish, the migration of immune cells showed decreased in primary and secondary immune response compare with initial day. As the same reaction seen in the migration of immune cells showed decreased in primary and secondary immune response.

Table.1 Character Analysis of *Catla catla* Exposed to Antigens

S. No	Character	Control	Whole cell bacterial antigen	Nucleotide antigen
1	Weight of the animal (gm)	26±2	25±2	25±3
2	Movement of the animal	Normal	Resting	Abnormal
3	Adulation days	26±2	25±2	20±1

Table.2 T cell Counts in Primary and Secondary Immune Response against Pathogens at Different Time Intervals

S. No.	Bacterial Strains	% of T cell production at different weeks							
		Primary Immune Response				Secondary Immune Response			
		Initial day	Week			Initial day	Week		
			I	II	III		I	II	III
1	Normal	64±2	62±2	61±2	63±2	64±2	62±2	62±2	64±2
2	Whole cell antigen	64±2	51±2	54±2	56±1	64±2	51±2	52±2	58±2
3	Nucleotide antigen	64±2	35±2	37±1	36±1	64±2	48±1	50±2	54±1

Table.3 Delayed Type Hypersensitivity in Primary and Secondary Immune Response against Pathogens at Different Time Intervals (HK)

S. No.	Bacterial Strains	Delayed type hypersensitivity at different times							
		Primary Immune Response				Secondary Immune Response			
		Initial day	Week			Initial day	Week		
			I	II	III		I	II	III
1.	Whole cell antigen	-	-	-	-	-	-	-	+
2.	Nucleotide antigen	+		+	+	+	+	+	+

+ Erytheme alone ++ Erythema with oedema - No significant change over control

Table.4 Lymphocyte Migration Assay in Primary and Secondary Immune Response against Pathogens at Different Time Intervals (WC)

S. No.	Bacterial Strains	Lymphocyte migration assay at different weeks (The values are measured in cm)							
		Primary Immune Response				Secondary Immune Response			
		Initial day	Week			Initial day	Week		
			I	II	III		I	II	III
1.	Whole cell antigen	1.2±0.2	1.1±0.2	0.9±0.2	0.7±0.1	1.2±0.2	0.9±0.2	0.7±02	0.6±0.1
2.	Nucleotide antigen	1.2±0.3	1.1±0.2	0.9±0.2	0.8±0.1	1.2±0.2	0.9±0.2	0.9±0.2	0.8±0.1

Ruscetti *et al.* (1993) reported that intravascular immune cells to the proximity of the infective focus and prepare them for extravasation controlled by adhesion molecules and chemokines contribute to cell migration and localization, as well as affect priming and differentiation of T cell responses. The migration of immune cells in

response to stimuli and the inhibition of immune cell migration by immunosuppressive factors. Cell migration and invasions are processes that after rich targets for intervention in key physiologic and pathologic phenomenon such as wound healing and cancer metasis (Friedl and Wolf, 2003). The results showed that migration of lymphocyte in primary and secondary immune response against whole cell bacterial antigen and Nucleotide antigen has inhibition of immune cell migration by immune suppressive factors.

Conclusion

Pseudomonas bacterial antigens such as whole cell antigen and nucleotide antigen was exposed fish showed the antibody level was increased to mediate the cell mediated immune response induce cytotoxic activity to pathogenic strains. The enhancement of this type of immune responses confirms the potential of bacterial antigen to be used as a vaccine.

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How to cite this article:

Thangaraj, A., G. Rekha and Dhasaratha, P. 2016. Cell Mediated Immune Response in a Fresh Water Fish, *Catla catla* against a Bacterial Antigen. *Int.J.Curr.Res.Aca.Rev.*4(3): 114-119. doi: http://dx.doi.org/10.20546/ijcrar.2016.403.013