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The immune response in catfish, *Mystus gulio*

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Abstract: *Light microscopic studies of the immune response of Mystus gulio were carried out. Antigen binding cells have been detected by plaque-forming cell (PFC) assay test in the spleen, head-kidney (HK) and thymus. Among these three organs, the HK is more pronounced in its response, and it is compared, on the basis of its histology of higher vertebrates. The peak response after primary and secondary immunizations was on day 7, in both circulating blood and the immune organs. The results suggest that HK in these fish might be the major organ for antibody secreting cells. The HA (haemoagglutinin) response was also for longer duration but only slightly more intense. The PFC response after the secondary immunization was for much longer duration and much more intense than after the primary immunization.*

Key words: *Immune response, Spleen, Thymus, Head-kidney, Plaque-forming cells, Mystus gulio*
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Introduction

All groups of lower vertebrates possess a well developed immunological capacity to respond to soluble and particulate antigen (Ortiz-Muniz and Sigel, 1971; Kanakambika and Muthukkaruppan, 1972; Kidder *et al.,* 1973; Sailendri and Muthukkaruppan, 1975; Corbel, 1975; Yamamoto *et al.,* 1980; Rijkers *et al.,* 1981; Nakanishi, 1982, 1985, 1986a; Plumb *et al.,* 1986; Chilmonczyk, 1991; Sinha and Chakravarty, 1997; Deivasigamani, 2007) though the diversity of the binding sites which is low when compared to that in mammals (Pastoret *et al.,* 1998; Fournier-Betz *et al.,* 2002).

The importance of the spleen of fish in immune response has been contradictory. Ontogenetic studies in salmon, (Ellis, 1977) and rainbow trout, *Salmo gairdneri* (Grace and manning, 1980) suggested that the spleen is not vital for immunological maturity since lymphocytes of thymus and kidney carry surface immunoglobulin and display mixed leucocyte reactions at a time when spleen is only present in a rudimentary form. On the other hand, the spleen of blue gourami, *Trichogaster trichopterus*, is supposed to be a major lymphoid organ (Yu *et al.,* 1970). The aim of this study is to evaluate the immune responses in the serum by haemoagglutinin test and in the three immune organs by the plaque-forming cell assay, of the estuarine catfish, *M. gulio* following immunization with sheep erythrocytes.

Materials and Methods

Adult catfish*, Mystus gulio* of both sexes weighing about 50-55 g and measuring 12-16 cm in standard length, selected at random from the stock, were used for the present study. Selected at season of September to March and temperature was approximately 23-25°C, at age of six month.

Antigen: Sheep blood was collected in Alsever's solution and washed three times in 0.85 percent saline by four successive centrifugations and the desired concentration of Sheep red blood corpuscles (SRBC) was prepared before using. All the experimental fish were immunized with 20 percent SRBC at a dose of 0.2 ml / fish, through intraperitoneal route. Again, a second dose of SRBC was given on day 20 after the primary immunization. Control fish were injected with equal volume of 0.85 percent saline. Immune responses in the serum , spleen, HK and thymus were assessed daily from day one to day 46. Six experimental and three control fish were sacrificed every day by using the method suggested by Sailendri and Muthukkaruppan (1975).

Haemagglutination: The haemagglutinin (HA) antibodies were detected in each animal by using two-fold dilutions of inactivated serum sample. The blood from each immunized and control fish was collected from the common cardinal vein, the serum extracted and inactivated at 56°C for 20 minutes. In a series of round bottom test tubes arranged vertically, double serial dilutions of the inactivated sera were made in 0.85 percent saline, to give a final volume of 0.5 ml. 0.250 ml of 20 percent SRBC suspension was added to each tube. This solution was mixed thoroughly and the tubes were incubated at room temperature for 1-2 hr. The highest reciprocal dilution of serum that caused complete agglutination of SRBC was noted as suggested by Sailendri and Muthukkaruppan (1975).

Plaque-forming cell assay: The plaque-forming cells (PFC) were detected in each animal by using the method suggested by Sailendri and Muthukkaruppan (1975). Organs like spleen, head kidney and thymus were dissected out from immunized and control fish. The cell suspensions from each of these organs were individually prepared in 1 ml of 0.85 percent saline. The concentration and viability of these cells were determined by the trypan blue dye exclusion method and by counting viable white cells in a haemocytometer. A reaction mixture consisting of 0.1 ml of the test cell suspension, 0.01 ml of fish complement and 0.01 ml of 20 percent SRBC was prepared and incubated as a monolayer between two glass slides for about 1 hr at room temperature. The incubated slides were observed under light microscope and scored for the number of hemolytic plaques. The number of PFC per million leucocytes was calculated.

Mean and standard deviation (X ± SD) were calculated for each set of the experimental data. One way analysis of variance and

Table - 1: Immune responses in *Mystus gulio* following intraperitoneal SRBC injection

S.No.	Days after immunization	PFC/10^6 white cells (Mean ± S.D.)			HA (Mean ± S.D.)
		Spleen	Head kidney	Thymus	
1	1	- - - -	- - - -	- - - -	- - - -
2	2	118.50 ± 3.93	130.83 ± 4.79	101.83 ± 1.9	1.33 ± 0.51
3	3	136.16 ± 8.81	155.66 ± 2.73	131.00 ± 6.8	1.50 ± 0.54
4	4	260.00 ± 4.89	271.33 ± 3.14	218.66 ± 9.7	3.33 ± 1.03
5	5	262.50 ± 11.22	288.33 ± 4.92	225.50 ± 4.6	3.00 ± 1.09
6	6	344.33 ± 4.76	381.00 ± 2.60	305.66 ± 4.8	10.00 ± 4.89
7	7	442.66 ± 4.58	492.33 ± 2.50	326.66 ± 3.7	24.00 ± 8.76
8	8	375.33 ± 10.9	391.33 ± 2.50	310.16 ± 5.3	6.66 ± 2.06
9	9	232.50 ± 1.87	253.50 ± 3.33	157.50 ± 5.7	3.00 ± 1.09
10	10	259.16 ± 5.56	271.00 ± 2.28	193.33 ± 5.4	6.00 ± 2.19
11	11	214.16 ± 3.18	236.16 ± 1.16	152.66 ± 2.33	1.33 ± 0.51
12	12	201.00 ± 1.26	212.00 ± 2.09	128.16 ± 1.9	1.50 ± 0.54
13	13	172.33 ± 2.06	176.66 1 2.58	91.33 ± 3.9	1.33 ± 0.51
14	14	143.83 ± 3.92	156.66 ± 5.85	103.16 ± 3.12	1.50 ± 0.54
15	15	115.00 ± 3.84	145.33 ± 4.45	103.66 ± 3.6	1.66 ± 0.51
16	16	101.33 ± 1.50	123.16 ± 1.94	91.83 ± 1.9	1.50 ± 0.54
17	17	94.50 ± 3.01	110.00 ± 1.41	94.50 ± 3.01	1.33 ± 0.51
18	18	86.33 ± 3.7	104.66 ± 5.00	77.00 ± 2.0	1.00 ± 0
19	19	- - - -	- - - -	- - - -	- - - -
20	20	- - - -	- - - -	- - - -	- - - -
21	21	- - - -	- - - -	- - - -	- - - -
22	22	113.83 ± 5.70	120.00 ± 8.09	93.66 ± 2.73	2.66 ± 1.03
23	23	156.83 ± 3.71	190.00 ± 3.98	141.16 ± 2.63	2.66 ± 1.03
24	24	245.00 ± 5.72	276.00 ± 4.33	165.83 ± 5.11	6.66 ± 2.06
25	25	313.00 ± 7.09	369.83 ± 7.08	223.33 ± 2.16	6.66 ± 2.06
26	26	390.00 ± 2.31	404.00 ± 5.09	296.5 ± 2.73	13.33 ± 4.13
27	27	696.00 ± 8.94	795.5 ± 4.08	332.5 ± 12.72	26.66 ± 8.26
28	28	639.00 ± 8.75	674.83 ± 4.66	311.33 ± 6.18	21.33 ± 8.26
29	29	515.50 ± 4.23	565.33 ± 11.48	266.00 ± 3.74	18.66 ± 6.53
30	30	435.50 ± 4.23	506.00 ± 3.74	235.33 ± 3.88	21.33 ± 8.26
31	31	292.5 ± 1.87	454.16 ± 3.31	252.16 ± 2.13	12.00 ± 4.38
32	32	311.66 ± 6.47	335.33 ± 3.01	231.83 ± 2.13	12.00 ± 4.38
33	33	288.33 ± 4.71	316.33 ± 3.14	215.5 ± 4.23	10.66 ± 4.13
34	34	246.16 ± 2.63	305.00 ± 3.57	205.00 ± 3.74	10.66 ± 4.13
35	35	239.00 ± 4.42	304.00 ± 6.29	198.83 ± 1.16	10.66 ± 4.13
36	36	229.16 ± 1.47	282.5 ± 2.50	183.66 ± 2.94	9.33 ± 3.26
37	37	211.5 ± 6.28	256.66 ± 4.08	172.33 ± 2.06	9.33 ± 3.26
38	38	188.16 ± 1.72	233.00 ± 2.36	152.83 ± 1.72	6.00 ± 2.19
39	39	163.00 ± 1.78	199.00 ± 0.89	119.83 ± 1.32	3.66 ± 0.81
40	40	146.00 ± 3.74	170.00 ± 4.60	99.5 ± 1.04	3.00 ± 1.09
41	41	147.5 ± 1.87	154.16 ± 1.94	94.33 ± 4.50	1.50 ± 0.54
42	42	134.00 ± 2.89	142.5 ± 1.87	81.16 ± 4.35	1.50 ± 0.54
43	43	123.83 ± 2.85	142.5 ± 1.37	66.16 ± 3.60	1.33 ± 0.51
44	44	104.16 ± 4.35	118.00 ± 1.41	55.00 ± 3.20	1.66 ± 0.40
45	45	89.16 ± 1.16	109.33 ± 6.91	58.5 ± 5.20	1.00 ± 0
46	46	- - - -	- - - -	- - - -	- - - -
F		**5564.55**	***8698.69**	**2226.87**	**24.69**
		p<0.001	**p<0.001**	**p<0.001**	**p<0.001**
Significant difference		**9.97**	***9.06**	**8.78**	**6.71**

*Significant values

Q-test (Snedecor and Cochron, 1967) were performed to assess the significance of the difference between means.

Results and Discussion

The results obtained in the adult catfish, *Mystus gulio* for the immune responses in the serum as assessed by HA titer in the spleen, HK, and thymus and by the PFC assay, against SRBC, are presented in the Table 1 and Fig. 1.

Haemagglutination: Serum antibodies were detected from the second day after the primary immunization of adult catfish, *M. gulio* with SRBC. The titer values increased gradually upto day 5, and

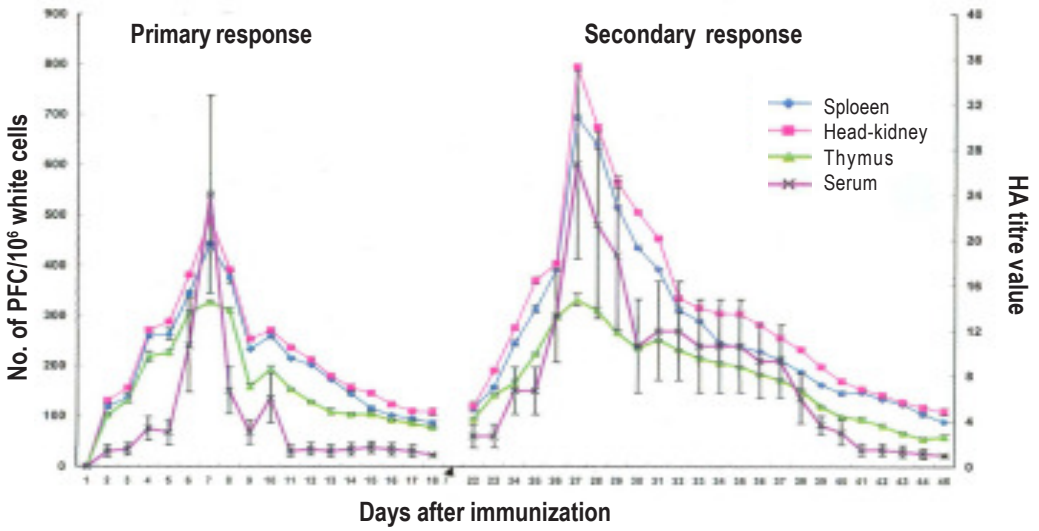

Fig. 1: Graph showing the kinetic of the immune responses in the serum and the three immune organs of *M. gulio* following immunization with 20 percent SRBC at a dose of 0.2 ml / fish. The secondary immunization was on day 20 (arrow). Each value is the mean of six measurements. Vertical bars represent standard deviation. HA value is the reciprocal of the dilution that showed the highest agglutination

significantly on days 6 and 7. The peak response was detected on the 7th day. A sudden significant decline in the antibody response ensued on day 8 and the response ended on the 11th day, touching the day 2 levels. Following secondary immunization on day 20 after the primary immunization, the serum immune response, as expressed by the HA titer values, rose somewhat more steeply than was observed after the primary immunization, and reached the peak on the 7th day. However, the peak response following secondary immunization (26.66 ± 1.03, day 27) was only slightly higher than that after the primary immunization (24.00 ± 8.76, day 7), and statistically not significant. However, the HA titer values after the peak remained higher for a longer duration, *i.e.*, about 6-7 days, after the secondary immunization, compared to those after the primary immunization when it was about only one day. The minimal titer values were reached in about 12 days after the peak.

Plaque-forming cell assay: The plaque-forming cells mainly small and large lymphocytes of the spleen, head-kidney and thymus of *M. gulio* were found from the second day after the primary immunization. The general trend in the rise and fall of the PFC count was similar in all the three immune organs and it was also similar to that of the HA titer values. The peak levels of PFC counts were detected on the 7th day and the minimal values on the 18th day after primary immunization. Among the three organs, the PFC count was always higher in the HK than in the spleen and thymus, the least being in the thymus, throughout the experimental period. The response of the PFC counts in the three organs following secondary immunization on day 20 was similar to that after the primary immunization, but more pronounced and prolonged. The peaks were after 7 days and pre-immunization levels were reached gradually in about 12 days after the peak.

The results obtained in the present study clearly demonstrated the capability of the freshwater catfish, *Mystus gulio* to develop humoral and cell-mediated immunity when challenged with SRBC. The immune responses were detected from day 2 after immunization, primary as well as secondary. A comparison of the humoral (HA) and cell-mediated (PFC) responses in the serum and in the three immune organs, respectively, following primary and secondary immunizations with SRBC is shown in Table 1. It could be seen from this table that: (1) the peak response was on day 7 after both primary and secondary immunization; (2) the PFC response after primary immunization lasted much longer in the HK (16 days) than in the spleen (15 days) and in the thymus (13 days); the HA response after the primary immunization was for the least duration (11 days); (3) the PFC response after the secondary immunization was for much longer duration and much more intense than after the primary immunization; the HA response was also for longer duration but only slightly more intense. Further, it could be noted, that the intensity of the peak response was always higher in the HK than in the spleen and in the thymus, the least intensity being thymus.

The comparison of results obtained in *M. gulio* with those in other species is rather difficult because of the dissimilarities in the immunization schedule and in other related factors. For instance, in *T. mossambica* Sailendri and Muthukkaruppan (1975) found intravenous administration of 0.5 ml of 20 percent SRBC to elicit maximum immune response when compared to intramuscular or intraperitoneal administration of similar or lesser dosages.

Dependence of antibody response on the concentration of antigen, dosage, and frequency of antigen administration was investigated in the rock fish, *S. marmoratus* (Nakanishi, 1982). The kinetics of the antibody responses in these immunized fish was studied by the appearance of circulating antibody and haemolysin plaque-forming cells, and it was found that the response was dependent on the antigen concentration, dosage and frequency of injection. A minimum antigen (5% SRBC) was needed to elicit immune responses. No significant difference was found among immune responses by the three routes of injection.

The effects of dose, route, number of injections and the use of adjuvant on the immune response in the brown trout, *Salmo trutta* have been studied (Ingram and Alexander, 1986). The primary and secondary immune responses were investigated in trout injected two or three times, with haemocyanin intramuscularly or intraperitoneally, with or without adjuvant and at different doses ranging from 1 to 20 mg. The route of injection significantly affected antibody production at low doses of antigen, intraperitoneal being faster than intramuscular.

The influence of route of administration on the humoral response of channel catfish *Ictalurus punctatus* to the particulate antigen, formalin-killed bacterin of *Yersinia ruckeri* has also been studied (Neumann and Tripp, 1986). The catfish were most responsive to the antigen (10^7 to 10^9 cells g^{-1}) when administered intramuscularly although lower doses (10^5 to 10^6 cells g^{-1}) given intraperitoneally elicited a substantial response. There was little evidence of dose dependent responses for any of the routes of immunization.

Seasonal changes in immune response, independent of the environmental temperature, have been correlated to sexual difference in relation to sexual maturity, in the rock fish (Nakanishi, 1986b). It was found in this fish that the reactivity of mature females to SRBC was lower than that of males or immature females in the spawning season (winter).

In the present study, the freshwater catfish used were adults of both sexes having small ranges of body weight and standard length. They were maintained at room temperature, which ranged between 28 and 30°C throughout the period of this study. The dosage of antigen administered was 0.2 ml of 20 percent SRBC and the route of injection was intraperitoneal. The choice of the dosage and route of injections was on the basis of their effectiveness and the facility with which they could be adopted with least amount of stress to the fish.

The peak response after primary and secondary immunizations was on day 7, in both circulating blood and the immune organs, in *M. gulio.* In the case of the *T. mossambica,* the peak HA titer was observed on day 11 and the peak PFC count on day 8, after the primary and secondary immunizations. On the other hand, in *C. batrachus,* the peak HA titer values after primary immunization was on day 10, and on day 7 after reimmunization. The PFC count was maximum on day 5 after both primary and secondary immunizations.

In conclusion, it may be said, that the freshwater catfish, *Mystus gulio* is endowed with humoral and cell-mediated immune capabilities, that all the three immune organs, *viz*., spleen, HK and thymus, are involved in antibody secretion, and that head-kidney is probably the major antibody producing organ.

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References

Chilmonczyk, S.: The thymus in fish : Development and possible function in the immune response. Annual Review of Fish Diseases. pp. 181-200 (1991).

Corbel, M.J.: The immune response in fish: A review. *J. Fish Biol.,* **7**, 539-563 (1975).

Deivasigamani, B.: Structure of immune organ in edible catfish, *Mystus gulio*. *J. Environ. Biol.,* **28**, 757-764 (2007).

Ellis, A.E.: The leucocytes of fish: A review. *J. Fish Biol.,* **11**, 453-491 (1977).

Fournier Betz, V., C. Quentel, F. Lamour and A. Leven: Immunocytochemical detection of Ig-positive cells in blood, lymphoid organs and the gut associated lymphoid tissue of the turbot (*Scophthalmus maximus*). *Fish Shell Fish Immunol.,* **10**, 2 (2002).

Grace, M.F. and M.J. Manning: Histogenesis of the lymphoid organs in rainbow trout, *Salmo gairdneri*. *Dev. Comp. Immunol.,* **4**, 255-264 (1980).

Ingram, G.A. and J.B. Alexander: The effect of dose, route, number of injections and the use of adjuvant on the clearance of and immune response to, haemocyanin following injection into brown trout, *Salmo trutta*. *Vet. Immunol. Immunopathol.,* **12**, 175-180 (1986).

Kanakambika, P. and V.R. Muthukkaruppan: The immune response to sheep erythrocytes in the lizard, *Calotes versicolor. J. Immunol.,* **109**, 415-419 (1972).

Kidder, G.M., L.N. Ruben and J.M. Stevens: Cytodynamics and ontogeny of the immune response of *Xenopus laevis* against sheep erythrocytes. *J. Embryol. Exp. Morph.,* **29**, 73-85 (1973).

Nakanishi, T.: The immune response of the rock fish, *Sebastiscus marmoratus*–I response of antibodies and hemolysin plaque–forming cells to SRBC. *Bull. Natl. Res. Inet. Aquaculture,* **3**, 81-89 (1982).

Nakanishi, T.: The immune response of the rock fish, *Sebastiscus maronoratus*-III. Eye transplantation immunity and effects of temperature on scale allograft rejection. *Bull. Natl. Res. Inst. Aquaculture,* **8**, 43-50 (1985).

Nakanishi, T.: Antibody producing cells in the marine telost, *Sebastiscus marmoratus*, organ distribution and morphology. *In*: European aquaculture society, Spec. Publ. (*Eds*.: C.P. Vivares, J.R. Bonami and E. Jaspers). 9, Bredene. pp. 333-342 (1986a).

Nakanishi, T.: Ontogenetic development of the immune response in the marine teleost *Sebastiscus marmoratus*. *Bull. Jap. Soc. Sci. Fish.,* **52**, 437-477 (1986b).

Neumann and Tripp: Influence of route of administration on the humoral response of channel catfish *Ictalurus punctatus* to *Yersinia ruckeri*. *Vet. Immunol. Immunopashol*., **12**, 163-174 (1986).

Ortiz Muniz, G. and M.M. Sigel: Antibody synthesis in lymphoid organs of two marine teleosts. *J. Rep. Endo. Soc.,* **9**, 42-52 (1971).

Pastoret, S.P.P., P. Griebel, H. Bazin and A. Govaerts: Immunology of fishes. *In*: Handbook of vertebrate immunology. Academic Press. London. pp. 3-62 (1998).

Plumb, J.A., M.L. Wise and W.A. Rogers: Modulary effects of temperature on antibody response and specific resistance to challenge of channel catfish, *Ictalurus punctatus* immunized against *Edwardsiella ictaturi*. *Vet. Immunol. Immunopathol.,* **12**, 297-304 (1986).

Rijkers, G.T., R. van. Oosterom and W.B. van. Muiswinkel: The immune system of cyprinid fish. Oxytetracycline and the regulation of humoral immunity in carp. *Vet. Immunol. Immunopathol.,* **2**, 281-290 (1981).

Sailendri, K. and V.R. Muthukkaruppan: The immune response of the teleost, *Tilapia mossambica* to soluble and cellular antigens. *J. Exp. Zool.,* **191**, 371-383 (1975).

Sinha, A. and A.K. Chakravarty: Immune responses in an air-breathing teleost, *Clarius batrachus*. *Fish Shell Fish. Immunol.,* **7**, 105-144 (1997).

Snedecor, G.W. and W.G. Cochran: Statistical methods, Oxford and IBH Publishing Co., New Delhi (1967).

Yu, M. L., D.A. Sarot, R.J. Filazzola and A. Perlutter: Effects of splenectomy of the immune response of the blue gourami, *Trichogaster trichopterus* to infectious pancreatic necrosis (IPN) virus. *Life Sciences.,* **9**, 749 (1970).

Yamamoto, K.I., Y. Itazawa and H. Kobayashi: Supply of erythrocytes into the circulating blood from the spleen of exercised fish. *Comp. Biochem. Physiol.,* **65**, 5-11 (1980).