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# Studies on Humoral Responses in *Mystus gulio* (Hamilton)

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**Abstract:** The haemagglutinin (HA) antibodies were detected in each animal by using two-fold dilutions of inactivated serum sample. Serum antibodies were detected from the second day after the primary immunization of adult *Mystus gulio* with SRBC. The titer values increased gradually upto day 5 and significantly on days 6 and 7. The peak response was detected on the 7th day. The minimal titer values were reached in about 12 days after the peak. The aim of this study is to evaluate the immune responses in the serum by haemoagglutinin test in freshwater catfish *M. gulio*.

**Key words:** *Mystus gulio*, humoral responses

## INTRODUCTION

All groups of lower vertebrates possess a well developed immunological capacity to respond to soluble and particulate antigen (Smith *et al*., 1967; Kanakambika and Muthukkaruppan, 1972; Kidder *et al*., 1973; Sailendri and Muthukkaruppan, 1975; Corbel, 1975; Rijkers *et al*., 1981; Sinha and Chakravarty, 1997; Romano *et al*., 2002; Zapata *et al*., 2006), though the diversity of the binding sites is low when compared to that in mammals (Pastoret *et al*., 1998).

After immunization with soluble and particulate antigen, fish most often produce antibodies with specificity and measurable affinity for the immunizing agent and these antibodies have biological properties such as agglutination, precipitation, complement fixation, opsonization and skin sensitization. The immune organs in fish, which are involved in the synthesis and secretion of anybodies in response to antigen, are the spleen, HK and thymus (Corbel, 1975; Sailendri and Muthukkaruppan, 1975; Rijkers *et al*., 1981; Sinha and Chakravarty, 1997; Pastoret *et al*., 1998; Romano *et al*., 2002; Zapata *et al*., 2006).

The antibody response of fish is generally affected by various factors such as the enviroumental temperature related to season, photoperiod, age and size sex and reproductive cycle of the fish. The effect of temperature has been extensively studied and found that warm temperatures produce higher titers than cold ones (Nakanishi, 1985; Sinha and Chakravarty, 1997). In addition, factors like dosage of antigen, route and frequency of administration and use of adjuvant have also been found to influence the antibody response in fish to varying degrees.

## MATERIALS AND METHODS

Live specimens of *Mystus gulio* (Hamilton) of the family bagridae were collected from Ogyampet estuary near Chennai. A total of 60 adult fishes of both sexes, weighing 40-45 g (standard length 11-15 cm), was used in the present study.

### Antigen

Sheep blood was collected in Alsevers solution and washed three times in 0.85% saline by four successive centrifugations and the desired concentration of SRBC was prepared before using.

**Immunization**

All the experimental fish were immunized with 20% SRBC at a dose of 0.2 mL $fish^{-1}$, 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% saline. Immune responses in the serum assessed daily from day one to day 46. Six experimental and three control fish were sacrificed every day.

**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 cardiual vein, the serum extracted and inactivated at 56°C for 20 min. In a series of round bottom test tubes arranged vertically, double serial dilutions of the inactivated sera were made in 0.85% saline, to give a fiual volume of 0.5 mL. 0.250 mL of 20% SRBC suspension was added to each tube. This solution was mixed thoroughly and the tubes were incubated at room temperature for 1-2 h. The highest reciprocal dilution of serum that caused complete agglutination of SRBC was noted.

**Statistical Analysis**

Mean and standard deviation (X±SD) were calculated for each set of the experimental data. One way analysis of variance and Q-test were performed to assess the significance of the difference between means.

## RESULTS

The results obtained in the adult *M. gulio* for the immune responses in the serum as assessed by HA titer assay, against SRBC, are shown in Table 1.

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

| Days after immunization | HA (Mean±SD) |
|---|---|
| 1 | - |
| 2 | 1.33±0.51 |
| 3 | 1.50±0.54 |
| 4 | 3.33±1.03 |
| 5 | 3.00±1.09 |
| 6 | 10.00±4.89 |
| 7 | 24.00±8.76 |
| 8 | 6.66±2.06 |
| 9 | 3.00±1.09 |
| 10 | 6.00±2.19 |
| 11 | 1.33±0.51 |
| 12 | 1.50±0.54 |
| 13 | 1.33±0.51 |
| 14 | 1.50±0.54 |
| 15 | 1.66±0.51 |
| 16 | 1.50±0.54 |
| 17 | 1.33±0.51 |
| 18 | 1.00±0.00 |
| 19 | - |
| 20 | - |
| 21 | - |
| 22 | 2.66±1.03 |
| 23 | 2.66±1.03 |
| 24 | 6.66±2.06 |
| 25 | 6.66±2.06 |
| 26 | 13.33±4.13 |
| 27 | 26.66±8.26 |

Table 1: Continued

| Days after immunization | HA (Mean±SD) |
|---|---|
| 28 | 21.33±8.26 |
| 29 | 18.66±6.53 |
| 30 | 21.33±8.26 |
| 31 | 12.00±4.38 |
| 32 | 12.00±4.38 |
| 33 | 10.66±4.13 |
| 34 | 10.66±4.13 |
| 35 | 10.66±4.13 |
| 36 | 9.33±3.26 |
| 37 | 9.33±3.26 |
| 38 | 6.00±2.19 |
| 39 | 3.66±0.81 |
| 40 | 3.00±1.09 |
| 41 | 1.50±0.54 |
| 42 | 1.50±0.54 |
| 43 | 1.33±0.51 |
| 44 | 1.66±0.40 |
| 45 | 1.00±0.00 |
| 46 | - |
| ANOVA; F | 24.69 p<0.001 |
| Q-test; Significant Difference | 6.71 |

**Haemagglutination**

Serum antibodies were detected from the second day after the primary immunization of adult *M. gulio* with SRBC. The titer values increased gradually upto day 5 and 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 (2.66±1.03) was only slightly higher than that after the primary immunization (24.00±8.76) 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.

## DISCUSSION

The results obtained in the present study clearly demonstrated the capability of the freshwater catfish *Mystus gulio* to develop humoral immunity when challenged with SRBC. The immune responses were detected from day 2 after immunization, primary as well as secondary. The peak response was on day 7 after both primary and secondary immunization and HA response after the primary immunization was for the least duration (11 days). The comparison of results obtained in *M. gulio* with those in other species is rather difficnlt because of the dissimilarities in the immunization schednle and in other related factors. For instance, in *T. mossmbica* (Sailendri and Muthukkaruppan, 1975), found intravenous administration of 0.5 mL of 20% 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 fish were immunized with SRBC at 23°C at different concentrations (20, 10, 5 and 1% SRBC at a dosage of 5 µL), at different dosages (25, 5 and 0.05 µL of 20% SRBC), through different routes (intraperitoneal, intravenous and subcutaneous) and at different frequencies ranging from a single

injection to three-time injection. The kinetics of the antibody responses in these immunized fish was studied by the appearance of circulating antibody and haemolysin plague-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 1-20 mg. The route of injection significantly affected antibody production at low doses of antigen, intraperitoneal being faster than intramuscular. For fish given more than two injections, the intramuscular route also gave significantly increased titers. The most important factor that affected the immune response to haemocyanin appeared to be the total amount of antigen injected, especially when given intraperitoneally with adjuvant.

The influence of route of administration on the humoral response of channel catfish *I. 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 $g^{-1}$ of fish) when administered intramuscularly although lower doses ($10^5$ to $10^6$ cells $g^{-1}$ of fish) given intraperitoneally elicited a substantial response. There was little evidence of dose dependent responses for any of the routes of immunization.

Effects of temperature on the humoral immune response of the above species of rock fish immunized with SRBC have also been studied (Nakanishi, 1985). Temperature independent seasonal modulations in antibody production have been reported in the perch *Perca fulviatilis* (Pontius and Ambrosius, 1972) in the rainbow trout *S. gairdneri* (Yamamoto *et al*., 1980) and in the rock fish *S. marmoratus* (Nakanishi, 1986b). In the case of the trout, those fish which were immunized with *Aeromonas salmonicida* prior to spring, showed much higher antibody levels than those which were immunized prior to winter, even when the animals were maintained at the same temperature of 18°C (Yamamoto *et al*., 1980). Antibody levels in the rock fish immunized with 20% SRBC (5 μL $g^{-1}$ body weight) in summer were higher than those in fish immunized in winter, even if the enviroumental temperature was held constant (Nakanishi, 1986a). These findings in the perch, trout and rock fish suggest the existence of seasonal factors other than temperature.

Seasonal changes in immune response, independent of the enviroumental 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). Further, exposure to long period of daylight was found to enhance the humoral response, especially in the adults than in the immature fish. These findings led to the suggestion of hormonal regulation in the humoral immune system.

Temperature related seasonal variations in immune response have been found in the air-breathing teleost *Clarias batrachus* (Sinha and Chakravarty, 1975). It was observed in this species that during winter (12-18°C), the number of PFC was much lower on day 5 after immunization with 0.2 mL of 25% SRBC, compared to the number observed in summer (28-32°C) and the rainy season (22-30°C).

The modulatory effects of various temperature treatments on the level of humoral antibody response in the chaunel catfish, *I. punctatus*, immunized with formalin-killed bacterial pathogen *Edwardsiella ictaluri*, were determined in laboratory controlled experiments (Plumb *et al*., 1986). Immunized fish that were held at 25°C for 30 days and at 12°C for an additional 30 days had higher antibody titers and were more protected upon challenge, than immunized fish held at 25°C for 60 days. Also immunized catfish held at 25°C for 5 or 10 days followed by 12°C water had higher antibody titers than immunized fish held at 12 or 25°C for 60 days. These results and those obtained in field

experiments carried out during winter and spring on fingerlings vaccinated using intraperitoneal injection or immersion with either sonicated or whole cell preparations of *E. ictaluri*, indicated that the channel catfish would develop humoral antibodies even if immunized in cold temperatures, as long as the fish are held in warm water for 4 or 5 days prior to cold water exposure. Data even strongly suggested that cool temperature is more desirable for maximum antibody level, longer antibody duration and better protection than continuous exposure to warm temperature.

The effect of the size and age on the functional capacity of the immune system has also been studied in the rock fish (Nakanishi, 1986b). Two month-old fish are able to mount an antibody response against SRBC (20%, 15 μL $g^{-1}$ body weight, intraperitoneal, at 23°C), while this capacity is lacking or very low in fry aged 1 month. The mean HA titer value increased with age. However, comparison between normal and dwarf rock fish revealed that immunological maturation was more dependent on the size rather than the age of the fish.

In spite of the differences among the various studies in fish in immunization schedule and other factors, discussed above, still it could be discerned that the general trend of the kinetics of the humoral immune responses of *M. gulio* to injected SRBC is comparable to those in several other species of teleost, especially those investigated in India (Sailendri and Muthukkaruppan, 1975; Sinha and Chakravarty, 1997).

In *M. gulio*, the onset of humoral antibody response was on within two days after primary immunization with SRBC, as was also observed in *T. mossombica* (Sailendri and Muthukkaruppan, 1975) and *C. batrachus* (Sinha and Chakravarty, 1997). 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. mossombica*, the peak HA titer was observed on day 11, 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 intensity and duration of immune response in *M. gulio* was higher following secondary immunization than after primary immunization, as also reported for *T. mossombica* and *C. batrachus*.

In conclusion, it may be said, that the freshwater catfish *Mystus gulio* is endowed with humoral immune capabilities are involved in antibody secretion.

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