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Immune Response of the Thymus to Sheep Red Blood Cells in White Spotted Char (*Salvelinus leucomaenis*)

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Abstract

White spotted char (*Salvelinus leucomaenis*), a salmonid fish, has been found to be able to elicit an immune response to sheep red blood cells (SRBC). The hemagglutinating titer (HA) markedly increased 12 days after the initial injection of SRBC (day 12) and then gradually decreased. The thymosomatic index (TSI, thymus weight (mg)/body weight (g) $\times$ 10^5) gradually increased 8 days after the initial injection of SRBC and sharply decreased on day 12.

The thymus of white spotted char consists of the cortex and the medulla. The cortex is deeply stained by hematoxylin. The medulla, intra-thymical area, is full of large lymphocytes and various epithelial cells. On day 12, an extreme involution of thymus occurred, and the number of large size lymphocytes markedly decreased.

Introduction

The T-cells (thymus-derived lymphocytes) are known to behave as a modulator of the immune system in mammals. Although teleost fishes have a well-developed immune system, the role of the thymus has been poorly understood[1].

Seasonal changes in the activities of thymus and other lymphoid organs have been studied in various fish species[2]. It is interesting to note that periodical changes of antibody titer against sheep red blood cells (SRBC) in *Sebastiscus marmoratus* were inversely related to the weight of the thymus, but not to the weight of the head kidney (pronephros)[3].

This study was conducted to find evidence on the role of the thymus in the immune system of a salmonid fish, white spotted char (*Salvelinus leucomaenis*).

Materials and Methods

Fish

Six-month-old white spotted char (*Salvelinus leucomaenis*), weighing 11.4 ± 0.6 g ($n = 10$), were obtained from Nanae Fish Cluture Experimental Station, Hokkaido University. The fish were reared at a constant temperature (15°C) on a 13L : 11D schedule for a month, and kept under the same condition throughout the experimental period.

Fish were anesthesized with a 1 : 1,000 aqueous solution of MS 222 (tricaine

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methanesulfonate) prior to bleeding. The blood was taken by caudal section. Serum was separated by centrifugation and stored at −20°C.

Immunization

SRBC in Alsever's solution were washed three times with 0.01 M phosphate buffer (pH 7.2) containing 0.15 M NaCl (PBS), and adjusted to 20% suspension in PBS. Five fish were given 3 intraperitoneal injections of 0.5 ml SRBC suspension at 2 day intervals. Five control fish received the same volume of PBS.

Hemagglutinating titers of anti-sera against SRBC were determined using a 96-well microtiter plate. Serial duplicate dilutions of test samples (25 μl) were made with gelatin veronal buffer (pH 7.5) containing 0.15 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (GVB^{++}). A 0.8% SRBC suspension in GVB^{++} was dropped into each well. The hemagglutinating activity was determined by the naked eye after incubation for 3 hr at 25°C.

Histological observation

The thymuses of both sides were dissected free of other tissues, weighed and fixed with Bouin's fluid for histological examinations. The thymosomatic index (TSI) was calculated as a ratio of the thymus weight to the body weight. The tissues were embedded in peraffin, and 6 μm serial sections were stained with hematoxylin and eosin (HE) by routine procedures.

Results

White spotted char elicited an antibody response against SRBC. The hemagglutinating titer of the experimental fish (n=5) increased from day 4 to day 12, where a peak value of 26.7±1.3 was observed. It gradually decreased to 11.3±1.3 on day 24. No antibody response against SRBC was detected in the sera from control fish (n=5) injected with PBS alone (Fig. 1). TSI was 28.5±2.1 at the start of the experiment, and reached a maximum value of 32.5±2.1 on day 8 (Fig. 2). It sharply dropped to 22.9±2.8 on day 12 and then tended to resume the initial level. No change in TSI was detected in control.

Histological observations of the thymus are shown in Fig. 3. The thymus of control fish was divided into two adjacent cell layers, the cortex and the medulla. The cortex was densely packed with small lymphocytes, and the medulla with large lymphocytes. On day 8, hypertrophy of the thymus was seen, but on day 12

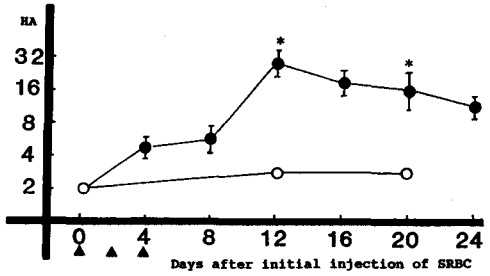

Fig. 1. Changes of antibody titer of white spotted char against SRBC. Closed circle (●) and open circle (○) represent the experimental and control groups, respectively. Arrows (▲) indicate injections of SRBC. HA (hemagglutinating titer) is represented as mean±S.D. (n=5). * Significantly different ($p<0.01$) from the control.

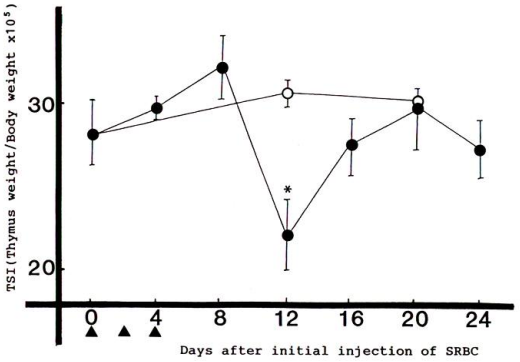

Fig. 2. Changes in thymosomatic index (TSI) of white spotted char immunized with SRBC. Closed circle (●) and open circle (○) represent experimental and control groups, respectively. Arrows (▲) indicate injections of SRBC. TSI is represented as mean±S.D. (n=5).
* Significantly different ($p<0.01$) from the control.

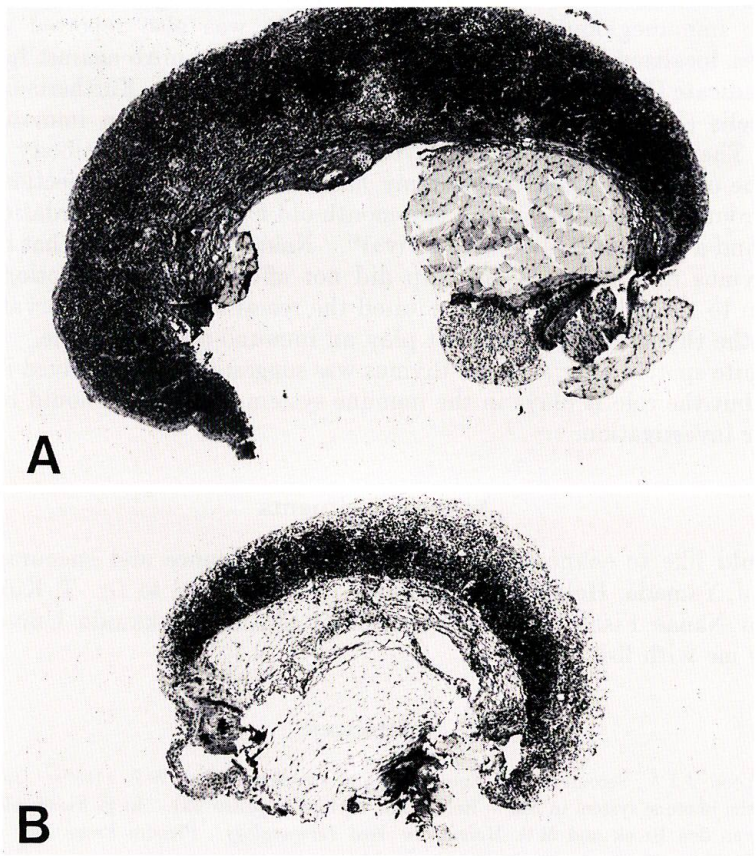

Fig. 3. Sections of the thymus of white spotted char before (A) and 12 days after (B) the immunization with SRBC. ×48.

atrophy of the thymus was observed with a concomitant decrease of large lymphocytes in the medulla. On day 16, the thymus resumed its initial size, but the distinction between the cortex and the medulla was not clear.

Discussion

This study has suggested that the thymus of white spotted char is involved in the immune response against SRBC. Two different roles of the thymus in the response can be postulated: one is that the antigen-binding activity and the other is an immuno-modulator function as seen in mammalian T-cells.

Ruben et al.[4] reported that antigen-binding cells (ABC), not antibody-producing cells, appeared in the thymus of goldfish immunized with SRBC, and that the number of ABC peaked earlier in the thymus than in the kidney or the spleen. Such a response to the antigen has not been found in the mammalian thymus. They also described that those antigen-binding cells in the thymus might be T helper cells[4]. Some mouse monoclonal antibodies (mAb) used against carp thymocytes reacted to immunoglobulin (Ig) in serum[5,6]. It was also reported that large thymocytes, localized in the medulla, were stained by the mAb against Ig[6]. These findings indicate that Ig molecules may occur in the thymus. Furthermore, plaque-forming cells (PFC) were found in the thymus of carp when immunized with SRBC[7]. Therefore, Ig in the carp thymocyte may possess an antibody activity.

On the other hand, the thymectomy had no effect on graft rejection in adult rainbow trout, but the operation in two-month-old fish caused a retardation of graft rejection and a prolonged allograft survival[8]. Nakanishi[9] reported that irradiation to the thymus in golden crucian carp did not affect allograft rejection, whereas irradiation to the spleen strongly inhibited the reaction. These observations indicate that the thymus of fish does not play an immuno-modulator role.

In white spotted char also, the thymus was suggested to be involved in immune response, but the role it plays in the immune system of this fish should be clarified by further investigation.

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References

1) van Loon, J.J.A., Secombes, C.J. Egberts, E. and van Muiswinkel, W.B. (1982). Ontogeny of the immune system in fish — Role of the thymus — p. 335-341. In P. Nieuwenhuis, A.A., van den Broek and M.G. Hanna, *In Vivo Immunology*. Plenum Press, New York and London.

2) Tamura, E. (1978). Studies on the morphology of the thymus in some Japanese fish. *Spec. Publ. Sado. Mar. Biol, Stat, Niigata Univ.* 1-75.

3) Nakanishi, T. (1986). Seasonal changes in the humoral immune response and the lymphoid tissuess of the marine teleost, *Sebastiscus marmoratus*. *Veterinary Immunol. Immunopathol.*

12, 213-221.

4) Ruben, L.N. Warr, G.W., Decker, J.M. and Marchalonis, J.J. (1977). Phylogenic origins of immune recognition: Lymphoid heterogeneity and the hapten/carrier effect in the goldfish, *Carassius auratus. Cell. Immunol.* **31**, 266-283.
5) Egberts, E., Secombes, C.J., Wellink, J.E., Groningen, J.J.M. and Muiswinkel, W.B. (1983). Analysis of lymphocyte heterogeneity in carp, *Cyprinus carpio* L., using monoclonal antibodies. *Dev. Comp. Immunol.* **7**, 749-754.
6) Secombes, C.J., van Groningen, J.J..M. and Egberts, E. (1983). Separation of lymphocyte subpopulation in carp *Cyprinus carpio* L. by monoclonal antibodies: immunohistochemical studies. *Immunology* **48**, 165-175.
7) Sailendri, K. and Muthkkaruppan, VR. (1975). The immune response of the teleost, *Tilapia mosambica* to soluble and cellular antigens. *J. Exp. Zool.* **191**, 371-382.
8) Manning, M.J., Grahe, M.F. and Secombes, C.J. (1982). Ontogenic aspects of tolerance and immunity in carp and rainbow trut: Studies on the role of the thymus. *Dev. Comp. Immunol.* Suppl. **2**, 75-82.
9) Nakanishi, T. (1986). Effect of partial-body irradiation on allograft rejection in the golden crucian carp, *Carassius auratus subsp. Bull. Natl. Res. Inst. Aquaculture* **9**, 51-58.