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Comparative Chemiluminescent Response of Phagocytes from Peripheral Blood, Head Kidney and Spleen of the Cultured Rockfish (*Sebastes schlegeli*)

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To compare the respiratory burst activity potential of the phagocytes isolated from head kidney, spleen, and peripheral blood in cultured rockfish (*Sebastes schlegeli*), chemiluminescent (CL) response analysis was performed. The phagocytes isolated from peripheral blood showed greater and faster CL response to the opsonized zymosan compared to that of the phagocytes isolated from kidney or spleen. This may imply a significant role of the blood phagocytes in defence mechanism of rockfish. The different responses found in the CL analysis among the phagocytes isolated from peripheral blood, kidney, and spleen may reflect differences in activation state or activity of phagocytes.

Key words: Chemiluminescent response, Phagocyte, Blood, Kidney, Spleen

Introduction

Teleost fish possess an efficient system for trapping and phagocytising antigens with monocytes, macrophages, and granulocytes (Fange, 1994). Although killing mechanisms of phagocytes are not well established in fish, it is clear that phagocytosis and the production of oxygen free-radicals via the respiratory burst are important defence mechanisms of host against microbial infection (Sharp and Secombes, 1993; Secombes, 1994).

In teleost, phagocytes isolated from head kidney, spleen, and blood have been widely used to analyze phagocytic and respiratory burst activity. However, little data are available on the comparative analysis of chemiluminescent (CL) response among phagocytes isolated from those three sources of tissue in fish.

The aim of the present work was to compare the potential of respiratory burst activity of the phagocytes isolated from head kidney, spleen, and blood in cultured rockfish (*Sebastes schlegeli*) using CL response analysis.

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Materials and Methods

Fish

Rockfish weighing 125 ± 16g were obtained from a local commercial farm. The fish were acclimated to a 500ℓ fiberglass tank supplied with aeration at 20℃ for 3 weeks prior to the experiment. Once a day a dry commercial pelleted rockfish diet was fed at 1% body weight. After acclimation for 3 weeks, 5 fish showing no signs of diseases were selected for the experiments.

Isolation of blood phagocytes

From each fish, 0.5 mℓ of blood was collected by a heparinized syringe, then, whole blood was withdrawn by an non-heparinized syringe. The collected heparinized-blood was immediately placed on a 34/51% Percoll (Sigma) density gradient and centrifuged at 400 g for 30 min at 4℃. The cells of interphase were collected and washed twice at 400 g for 5 min in Hanks balanced salt solution (HBSS, Sigma) containing heparin and antibiotics. The cell viability was examined with trypan blue exclusion and was evaluated to be greater than 95%. The phagocytes were adjusted to 5×10^5 cells/mℓ HBSS.

Isolation of kidney and spleen phagocytes

Each kidney and spleen removed aseptically was

passed through a 100 μm nylon mesh using minimum essential medium (MEM, Sigma) containing heparin (10 units/mℓ, Sigma), penicillin (100 μg/mℓ Sigma) and streptomycin (100 U/mℓ, Sigma). The resulting cell suspension was placed on a 34/51% Percoll density gradient and centrifuged at 400 g for 30 min at 4℃. The next procedures were the same as those described above for the isolation of blood phagocytes.

Opsonization of zymosan

Zymosan (Sigma) was mixed with the serum of rockfish and incubated at 30℃ for 30 min. The opsonized zymosan was separated by centrifugation, washed three times and suspended in HBSS.

Chemiluminescence (CL) assay

The ROIs (reactive oxygen intermediates) produced by stimulated phagocytes was quantified using an automatic photoluminometer (Bio-Orbit 1251, Sweden). Each test cuvette contained 0.7 mℓ luminol (Sigma) prepared according to the method of Scott and Klesius (1981), 0.5 mℓ cell suspension, and 0.3 mℓ opsonized zymosan, which was added just prior to measurement. The measurement was done for 100 min and the light emission was recorded as mV.

Statistical analysis

The results were analysed by using the Student's t-test, $P < 0.05$ being taken as the minimum significance level.

Results

Peak value of chemiluminescent (CL) response

The phagocytes isolated from peripheral blood and spleen showed significantly ($P < 0.05$) higher CL peak value than that found in the phagocytes isolated from kidney (Fig. 1). The CL value of blood phagocytes was higher than that of spleen phagocytes, but there was no statistical difference.

Time to peak CL response

The required time to get the peak value of CL was significantly ($P < 0.01$) shorter in the phagocytes isolated from blood than that of phagocytes isolated from kidney (Fig. 2). Although the peak time of phagocytes isolated from spleen showed shorter than kidney phagocytes, and longer than that of blood phagocytes, significant differences were not found.

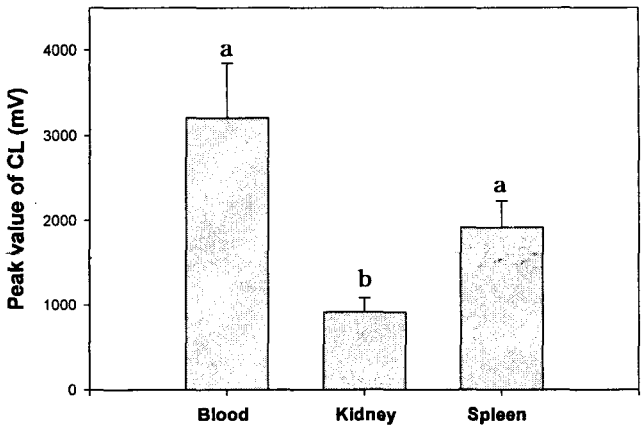

Fig. 1. The peak values of chemiluminescent response of the phagocytes isolated from peripheral blood, kidney and spleen in rockfish. Values are mean ± S.E. and different letters indicate statistical significance at $P < 0.05$.

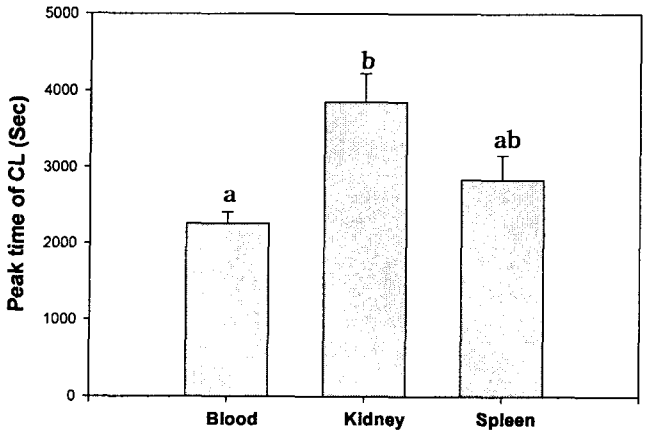

Fig. 2. Time to get peak value of chemiluminescent response of the phagocytes isolated from peripheral blood, kidney and spleen in rockfish. Values are mean ± S.E. and different letters indicate statistical significance at $P < 0.01$.

Discussion

Fish, like other vertebrates, respond to infectious pathogens in specific and non-specific ways. However, the non-specific defences are the first a pathogen encounters, and it has been suggested that they are very important in the resistance of fish to infectious agents (Blazer, 1991). Granulocytes and macrophages possess a phagocytic activity which is the initial step in the immune response in fish, and

is the major line of defence for all foreign materials, including pathogenic agents (Olivier et al., 1986). During phagocytosis, fish phagocytes increase their oxygen consumption as well as the production of reactive oxygen intermediates (ROIs) (Chung and Secombes, 1988) such as the superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH^-). These ROIs play an important role in the antimicrobial activity of phagocytic cells (Allen et al., 1972; Babior, 1984). Chemiluminescent (CL) response measures the respiratory burst activity of phagocytic cells in which oxygen is converted into ROIs.

In the present study, phagocytes isolated from peripheral blood showed greater and faster CL response to the opsonized zymosan compared to that of the phagocytes isolated from kidney or spleen. This may imply a significant role of the blood phagocytes in defence mechanism of rockfish.

The different responses found in the CL analysis among the phagocytes isolated from peripheral blood, kidney, and spleen may reflect differences in activation state or activity of phagocytes. When a pathogen gains entry to the tissues of a host, a common acute inflammatory response is elicited, and it can be characterized by neutrophilia and monocytosis in the blood, and an accumulation of neutrophils and macrophages at the site of infection (Suzuki and Iida, 1992). In this response, the increase blood neutrophils and their extravasation preceed the other responses (Ellis, 1986). This fact strongly suggests that blood phagocytes are more readily activated or have stronger activity than the phagocytes in kidney or spleen. The significantly higher CL response of spleen phagocytes than that of kidney phagocytes of the present study, also, indicates that spleen phagocytes have a more powerful respiratory burst potential than kidney phagocytes.

Walsh and Luer (1998) reported that cells isolated from epigonal and Leydig organs in two species of elasmobranch fish, the nurse shark (*Ginglymostoma cirratum*) and the clearnose skate (*Raja eglanteria*), had the greatest phagocytic and pinocytic activity in vitro compared to cells isolated from peripheral blood and spleen. The result of this study, also, showed that phagocytes isolated from different oragans in teleost fish had different potential in immune defense like elasmobranch.

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References

Allen, R.C., R.L. Stjernholm and R.H. Steele. 1972. Evidence for generation of an electronic excitation state(s) in human polymorphonuclear leucocytes and its participation in bactericidal activity. Biochem. Biophy. Res. Comm., 47, 679~684.

Babior, B.M. 1984. Oxidants from phagocytes: agents of defense and destruction. Blood, 64, 959~966.

Blazer, V.S. 1991. Piscine macrophage function and nutritional influences: a review. J. Aqua. Anim. Health, 3, 77~86.

Chung, S. and C.J. Secombes. 1988. Analysis of events occurring within teleost macrophage during the respiratory burst. Comp. Biochem. Physiol., 89B, 539~544.

Ellis, A.E. 1986. The function of teleost fish lymphocytes in relation to inflammation. Int. J. Tiss. React., 8, 263~270.

Fange, R. 1994. Blood cells, haemopoiesis and lymphomyeloid tissues in fish. Fish Shellfish Immunol., 4, 405~411.

Olivier, G., C.A. Eaton and N. Campbell. 1986. Interaction between *Aeromonas salmonicida* and peritoneal macrophages of brook trout (*Salvelinus fontinalis*). Vet. Immunol. Immunopathol., 12, 223~234.

Scott, A.L. and P.H. Klesius. 1981. Chemiluminescence: A novel analysis of phagocytosis in fish. Develop. Biol. Standard., 49, 243~254.

Secombes, C.J. 1994. Enhancement of fish phagocyte activity. Fish Shellfish Immunol., 4, 421~436.

Sharp, G.J.E. and C.J. Secombes. 1993. The role of reactive oxygen species in the killing of bacterial fish pathogen *Aeromonas salmonicida* by rainbow trout macrophages. Fish Shellfish Immunol., 3, 119~129.

Suzuki, Y. and T. Iida. 1992. Fish granulocytes in the process of inflammation. Annu. Rev. Fish Dis., 2, 149~160.

Walsh, C.J. and C.A. Luer. 1998. Comparative phagocytic and pinocytic activities of leucocytes from peripheral blood and lymphomyeloid tissues of the nurse shark (*Ginglymostoma cirratum* Bonaterre) and the clearnose skate (*Raja eglanteria* Bosc). Fish Shellfish Immunol., 8, 197~215.