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Immune response and disease resistance of *Oreochromis mossambicus* to *Aeromonas hydrophila* after exposure to hexavalent chromium

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ABSTRACT: The objective of this study was to investigate the effect of chronic exposure to sublethal concentrations of hexavalent chromium ($K_2Cr_2O_7$) on the immune response and disease resistance of *Oreochromis mossambicus* (Peters) to bacterial *Aeromonas hydrophila* infection. Fish (45 to 50 g) were exposed to 0.005, 0.05, 0.5, and 5 mg l^{-1} [0.01, 0.1, 1, and 10% LC_{50}, respectively] of hexavalent chromium Cr (VI) for 28 d. The specific immune response was assessed by antibody response to *A. hydrophila* by bacterial agglutination assay, and to sheep red blood cells (SRBC) by plaque forming cell (PFC) assay. In addition, nonspecific immune mechanisms were assessed by serum lysozyme activity and reactive nitrogen intermediates, the latter in terms of nitric oxide (NO) production by peripheral blood leucocytes. Overall immunity was assessed by disease resistance against live virulent *A. hydrophila.* The study clearly indicated that chronic exposure of fish to 0.5 and 5 mg l^{-1} of chromium (VI) decreased both nonspecific and specific parameters of the immune system, which resulted in a lower disease resistance to *A. hydrophila*. Interestingly, 0.05 mg l^{-1} of Cr (VI) enhanced disease resistance and both nonspecific and specific immune responses to *A. hydrophila*. Our study revealed a concentration-dependent modulation of the immune system by chromium (VI), as demonstrated by suppressive or stimulatory effects on lymphocytes, lysozyme, phagocytic killing mechanisms, and disease resistance in *O. mossambicus*.

KEY WORDS: Chromium · Exposure · Disease resistance · Lysozyme · Nitric oxide · *Oreochromis mossambicus* · Antibody response

INTRODUCTION

The aquatic environment of fish is influenced by a number of factors of both anthropogenic and natural origin. Many of these factors ultimately affect the fish homeostatic mechanism and thereby lead to energy exhaustion. This in turn negatively affects the energy-demanding process of immunity and increases susceptibility to disease. Chromium is one of the highly prevalent industrial pollutants in developing countries such as India (Khwaja et al. 2001). The most significant anthropogenic sources of chromium in waterways are leather tanning industries, electroplating operations, and textile manufacturing industries (ATSDR 2000). Among these, the leather-tanning industry is the major source of chromium deposition in Indian waterways (Khwaja et al. 2001). Almost 90% of tanning units in India are situated along the banks of rivers, where the concentration of hexavalent chromium has been found to range from 3.0 to 11.2 mg l^{-1}, far in excess of the permissible limit of 0.5 mg l^{-1} (Dubey et al. 2001, Koteswari & Ramanibai 2003, Tare et al. 2003).

In the aqueous environment, chromium has 2 ionic forms: hexavalent chromium Cr (VI) and trivalent chromium Cr (III). The former is widely used in industrial processes and is more toxic than Cr (III) (Blasiak &

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Kowalik 2000). Cr (VI) readily penetrates cell membranes and becomes reduced to more stable Cr (III). The reduction process of Cr (VI) to Cr (III) results in the formation of reactive intermediates that may cause DNA damage, membrane damage, and altered gene expression (Ye et al. 1999). On the other hand, Cr (III) is an essential component of human and animal nutrition and is required for energy metabolism (Anderson 1997). Several studies have investigated the effect of Cr (VI) on the immune system of different fish species. Chromium has been shown to suppress the humoral immune response against the viral MS2 bacteriophage in *Salmo trutta* L. and *Cyprinus carpio* L. (O'Neill 1981). Khangarot et al. (1999) have shown that exposure of the freshwater catfish *Saccobranchus fossilis* to sublethal levels of Cr (VI) compromises the humoral and cell mediated immunity by decreasing levels of antibody and splenic plaque forming cell number, by reducing proliferation of splenic lymphocytes, and by decreasing disease resistance to bacterial infection.

Chromium has also been found to suppress humoral immunity in *Oreochromis mossambicus* (Arunkumar et al. 2000). Reports on the effect of Cr (VI) on the nonspecific immune mechanisms of fish are very sparse. Cr (VI) affects various components of the immune system by immunosuppression or immunostimulation in both Wistar rats (Glaser et al. 1985) and humans (Borella et al. 1990). Furthermore, several reports demonstrate that dietary supplementation of Cr (III) or (VI) enhances immune responses in dairy cows by enhancing serum immunoglobulin production and antibody titres to antigens or vaccines, and by reducing serum cortisol concentration (Moonsie-Shageer & Mowat 1993, Chang et al. 1996). The objective of our study was to investigate the effect of chronic exposure to sublethal concentrations of Cr (VI) on disease resistance and nonspecific and specific immune responses in *O. mossambicus.*

MATERIALS AND METHODS

Fish. *Oreochromis mossambicus*, procured from a local farmer, were acclimated to ambient, uncontrolled laboratory water temperature (28 ± 2°C) under natural photoperiod for 2 wk in 165 l fiber reinforced plastic tanks. All tanks were fitted with a separate water recirculation system with external biofilters (Eheim-2213) with a flow rate of 7 l min^{-1}. Male fish weighing 45 to 50 g were used in the present study. All fish were fed once a day ad lib. with a balanced diet (protein: 39%; carbohydrate: 24%; lipid: 11%; water: 10%; ash: 9%; trace amounts of vitamins and minerals) prepared in the laboratory. Physico-chemical characteristics of the water were monitored and maintained.

Chromium exposure. Groups of fish were exposed to 0.005, 0.05, 0.5, or 5 mg l^{-1} (0.01, 0.1, 1, or 10% LC_{50}) of Cr (VI) for 28 d. After this period, fish continued to be held in chromium spiked water until sampling was completed. The specific immune response was assessed by antibody response to *Aeromonas hydrophila* using bacterial agglutination assay, and by number of antibody producing cells against SRBC using plaque forming cell (PFC) assay. To ascertain the complete primary antibody response, fish were sampled until Day 56 (until Day 28 post immunization). For PFC assay, fish were sampled until Day 43 (until Day 15 post immunization). Nonspecific immune mechanisms were assessed by serum lysozyme activity and reactive nitrogen intermediates, via assessment of nitric oxide (NO) production by peripheral blood leucocytes. To ascertain the nonspecific parameters, fish were sampled until Day 38. Overall immunity was assessed as the degree of resistance against live virulent *A. hydrophila*.

Serum separation. For serum separation, 200 µl of blood was drawn from the cardinal vein (Michael et al. 1994) and collected in serum tubes. Blood was allowed to clot overnight at 20°C, and was then centrifuged at $400 \times g$ for 10 min and the serum was separated. Serum was stored in sterile eppendorf tubes at –20°C until further use. For bacterial agglutination assay, the serum was de-complemented by incubation at 47°C for 30 min in a water bath (Sakai 1981).

Bacterial agglutination assay. For detecting the effect of chromium exposure on antibody response, 8 fish per group were maintained. Fish were administered intraperitoneally with heat killed *Aeromonas hydrophila* bacterin, containing 1×10^8 cells in 0.2 ml of phosphate buffered saline (PBS) per fish (Karunasagar et al. 1997) after 28 d of chromium exposure. An unimmunized control was also maintained. All fish were repetitively bled at regular intervals of 7 d post immunization until Day 28. Antibody titres against heat killed *A. hydrophila* were performed in 96 well 'V' bottom microtitre plates after Sakai et al. (1993) with slight modifications: 25 µl of serum was added to the first well and 2-fold serial dilutions were made with PBS, before 25 µl of heat killed *A. hydrophila* suspension (1×10^9 cells ml^{-1}) pre-stained with crystal violet was added to each well. The microtitre plate was incubated at 37°C overnight. The highest dilution of serum sample that showed detectable macroscopic agglutination was recorded and expressed as $\log_2$ antibody titre of the serum (Roberson 1990).

Plaque forming cell (PFC) assay. To study the effect of chronic exposure to Cr (VI) on the number of antibody producing cells, 15 fish $group^{-1}$ were maintained. All fish were primed (injected) intraperitoneally with 0.1 ml of 5% SRBC after 28 d of exposure. A booster dose of

0.1 ml of 25% SRBC was then administered through the same route after 3 d (Venkatalakshmi & Michael 2001). The direct PFC assay (Anderson 1990) was performed to determine the number of PFC. Five fish group^{-1} were killed on Days 5, 10, and 15 post immunization using excessive dose of MS-222, and the spleen was aseptically excised. By forcing the spleen through 100 µm nylon mesh, spleen cells were isolated. Cells were washed in RPMI 1640 and adjusted to 1×10^7 cells ml^{-1}. From the cell suspension, 100 µl were mixed in a serological tube along with 200 µl of 10% SRBC in RPMI 1640, 100 µl of 1.5% solution of warm (45°C) agarose and 100 µl of RPMI 1640 media, and were then poured immediately on to a glass microscope slide. Slides were incubated at 28°C for 4 h, then 500 µl of 10% guinea pig serum was added as a source of complement. Slides were further incubated for 2 h at 28°C. The numbers of PFC per slide were enumerated by observation under a dissection microscope at 5× magnification.

Serum lysozyme assay. For the lysozyme assay, 8 fish group^{-1} were maintained. All fish were repetitively bled at regular intervals of 2 d until Day 38, beginning after 28 d of chromium exposure. A serum sample had also been taken 2 d prior to chromium exposure. Serum lysozyme assay was determined using a turbidimetric assay developed by Parry et al. (1965) with the microplate adaptation of Hutchinson & Manning (1996). A suspension of 0.3 mg ml^{-1} of *Micrococcus lysodeikticus* in 0.05 M sodium phosphate buffer (pH 6.2) was used as substrate; 10 µl of serum was added to 250 µl of the bacterial suspension, and reduction in absorbance at 490 nm was determined after 0.5 and 4.5 min of incubation at 28°C in a microplate reader (Model 680, Biorad). One unit of lysozyme activity was defined as a reduction in absorbance of 0.001 min^{-1} (Ellis 1990).

Preparation of peripheral blood leucocytes. Blood was collected by cardinal vein puncture (Michael et al. 1994) into syringes filled with blood collecting medium (RPMI 1640 supplemented with 5×10^4 IU l^{-1} sodium heparin, 10^5 IU l^{-1} penicillin, and 100 mg l^{-1} streptomycin). Peripheral blood leucocytes (PBL) were separated from erythrocytes by centrifugation (800 × *g*, 20 min) over Lymphoprep (Nycomed) as described by Miller & McKinney (1994). Cell suspensions were washed 3 times with washing medium (RPMI 1640 supplemented with 10^5 IU l^{-1} sodium heparin, 10^5 IU l^{-1} penicillin, and 100 mg l^{-1} streptomycin) for 10 min at 700 × *g* and resuspended in culture medium (RPMI 1640 supplemented with 3% [v/v] of pooled, heat inactivated (30 min, 47°C) tilapia serum, 10^5 IU l^{-1} penicillin, 100 mg l^{-1} streptomycin, and 4 mM L-glutamine) (all chemicals: Biochrom AG). Numbers of viable cells (exclusion of trypan blue) were enumerated and adjusted to 4×10^7 ml^{-1} using cell culture medium.

Reactive nitrogen intermediates production assay. To study the effect of chronic exposure to Cr (VI) on the reactive nitrogen intermediates (RNI) in terms of nitric oxide (NO) production, 5 fish were maintained. Fish were exposed to different concentrations of Cr (VI) for 28 d. After this period, NO production was estimated by using peripheral blood leucocytes at regular intervals of 2 d until Day 38. Preparation of leucocytes was performed as described before. The release of NO by peripheral blood leukocytes in the medium was measured using Griess reaction. The Griess reagent indicates the presence of nitrite as a surrogate of NO (Green et al. 1982). Peripheral blood leukocytes were cultured for 96 h at 28°C and 50 µl culture supernatant was collected and transferred to a separate microtitre plate; 50 µl of Griess reagent (1% sulphanilamide, 0.1% N-Naphthyl-ethylenediamine, 2.5% phosphoric acid) was then added to each well containing the culture supernatant. After incubation for 10 min, the optical density was recorded spectrophotometrically at 570 nm. Molar concentrations of NO_2 were calculated from a standard curve generated from a graded series of $NaNO_2^-$ concentrations in culture medium.

Disease resistance test. To study the effect of Cr (VI) on disease resistance, groups of fish (n = 30 group^{-1}) were exposed to 0.005, 0.05, 0.5, or 5 mg l^{-1} of Cr (VI) for 28 d. These groups (in duplicate) were experimentally challenged with LD_{30} dose (0.2 ml PBS containing 1×10^8 cells) of live virulent *Aeromonas hydrophila*. Previously, fish had been administered a wide range of doses of *A. hydrophila* (ranging from 1×10^5 to 1×10^9 cells 0.2 ml^{-1} PBS) to determine the rate of mortality over time. Mortality was observed until Day 28 post infection, but no mortality was observed after 96 h of administration. Thus, in this experiment, time of exposure to the LD_{30} dose was set at 96 h. The average of the duplicate set was used to express percent mortality.

Statistics. Data were expressed as arithmetic mean ± SE. Student's 2-tailed *t*-test and 1-way analysis of variance (ANOVA) were used to test significant differences between and among groups, respectively, using Sigmastat 2.0 (Jandel Corporation). Differences were considered significant when $p < 0.05$.

RESULTS

Effect of Cr (VI) on antibody response

While the antibody response expressed as $\log_2$ of the agglutination titre was comparable among all groups 7 d after immunization, on Day 14 post immunization clear differences were evident (Fig. 1). A significant reduction compared to the control was found in groups previously exposed to 0.5 mg l^{-1} or 5 mg l^{-1} of Cr (VI)

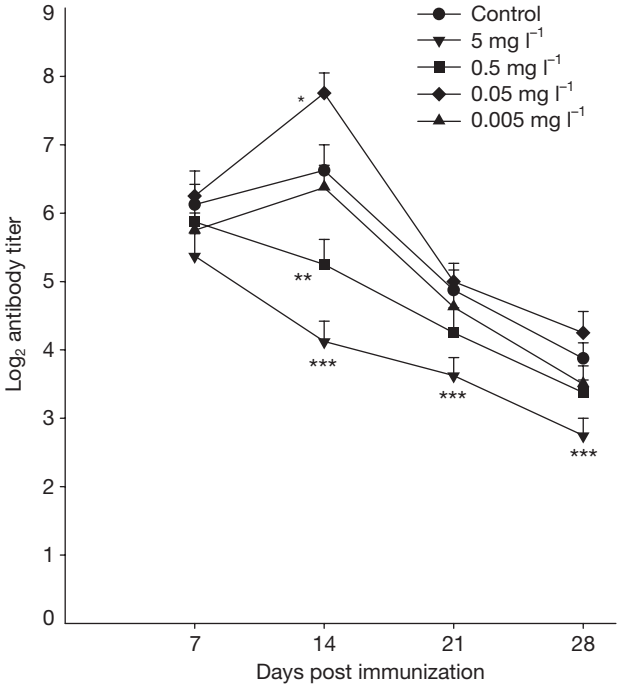

Fig. 1. *Oreochromis mossambicus*. Effect of chronic exposure to Cr (VI) on antibody response to heat killed *Aeromonas hydrophila*, assayed by bacterial agglutination. Points represent mean + SE; n = 8 group^{-1}; *p < 0.05; **p < 0.02; ***p < 0.01

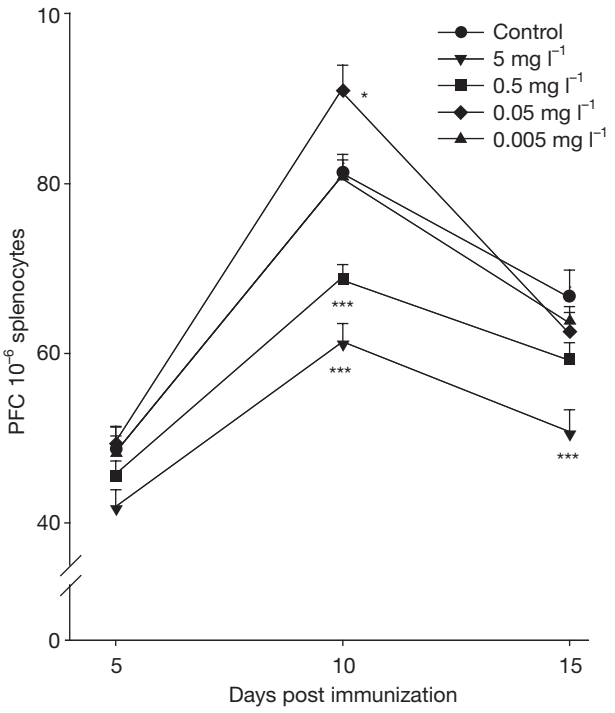

Fig. 2. *Oreochromis mossambicus*. Effect of chronic exposure to Cr (VI) on number of plaque forming cells (PFC). Points represent mean + SE; n = 5 group^{-1}; *p < 0.05; **p < 0.02; ***p < 0.01

($p < 0.05$ and $p < 0.01$, respectively), while the titre significantly increased in the group exposed to 0.05 mg l^{-1} of Cr (VI) ($p < 0.05$). On Day 21 and 28, fish exposed to 5 mg l^{-1} of Cr (VI) showed a significant ($p < 0.01$) suppression of antibody titre.

Effect of Cr (VI) on PFC numbers

The number of antibody producing cells visualized as PFC were comparable among all groups 5 d after SRBC injections (Fig. 2). However, on Day 10 post SRBC injection, clear differences were evident among groups. A significant ($p < 0.01$) reduction in PFC numbers compared to the control was observed in groups previously exposed to 0.5 mg l^{-1} or 5 mg l^{-1} of Cr (VI), while the PFC number significantly increased in the group exposed to 0.05 mg l^{-1} of Cr (VI) ($p < 0.05$). On Day 15, the group exposed to 5 mg l^{-1} of Cr (VI) showed a significant ($p < 0.01$) reduction in PFC number.

Effect of Cr (VI) on serum lysozyme activity

Serum lysozyme activity was comparable among all groups on Day 30, while exposure to 5 mg l^{-1} of Cr (VI) significantly decreased the serum lysozyme activity on all the other days tested ($p < 0.01$) (Fig. 3). A significant

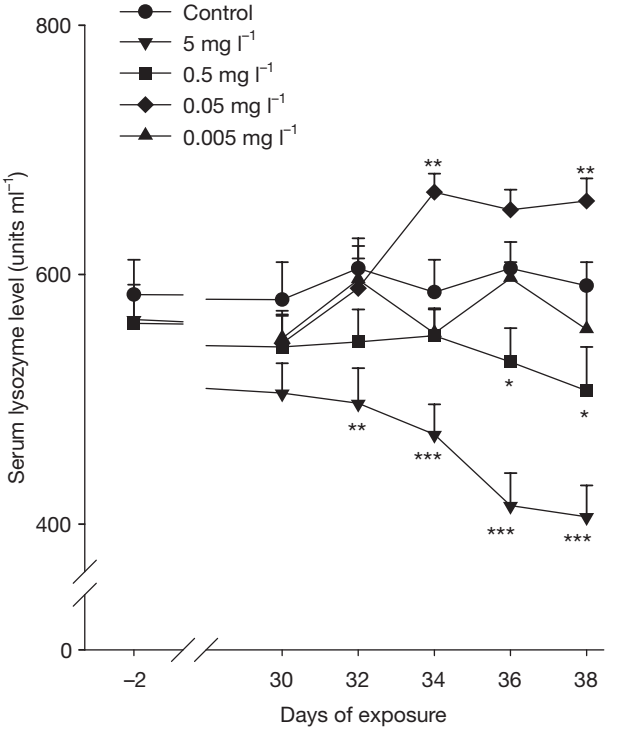

Fig. 3. *Oreochromis mossambicus*. Effect of chronic exposure to Cr (VI) on serum lysozyme level. Points represent mean + SE; n = 8 group^{-1}; *p < 0.05; **p < 0.02; ***p < 0.01

reduction in serum lysozyme activity on Days 36 and 38 was also observed in the group exposed to 0.5 mg l^{-1} of Cr (VI) ($p < 0.05$). Exposure to 0.05 mg l^{-1} of Cr (VI) resulted in significant ($p < 0.02$) enhancement of serum lysozyme activity on Day 34 and 38.

Effect of Cr (VI) on RNI production

The RNI was measured as NO production by peripheral blood leucocytes (Fig. 4). Significant reduction in NO production was observed on Day 30 and 38 ($p < 0.01$ and $p < 0.05$, respectively) in the group exposed to the highest concentration, i.e. 5 mg l^{-1} Cr (VI). Exposure to 0.5 mg l^{-1} of Cr (VI) significantly ($p < 0.05$) reduced the production only on Day 30. Exposure to lower concentrations (0.05 mg l^{-1} and 0.005 mg $l^{-1)}$ did not have any significant effect on the NO production on any day of testing.

Effect of Cr (VI) on disease resistance to *Aeromonas hydrophila*

Disease resistance was expressed as 96 h percent mortality. Fish exposed to higher concentrations of Cr (VI) (either 5 or 0.5 mg l^{-1}) showed increased percent mortality (75.9 and 56.1%, respectively) when administered with *Aeromonas hydrophila* (Fig. 5). Interestingly, fish exposed to 0.05 mg l^{-1} of Cr (VI) exhibited decreased percent mortality (13.2%) when compared to control fish not exposed to Cr (VI) (percent mortality 33.33%). However, the lowest concentration of Cr (VI) tested (0.005 mg l^{-1}) had no effect on percent mortality compared to the control.

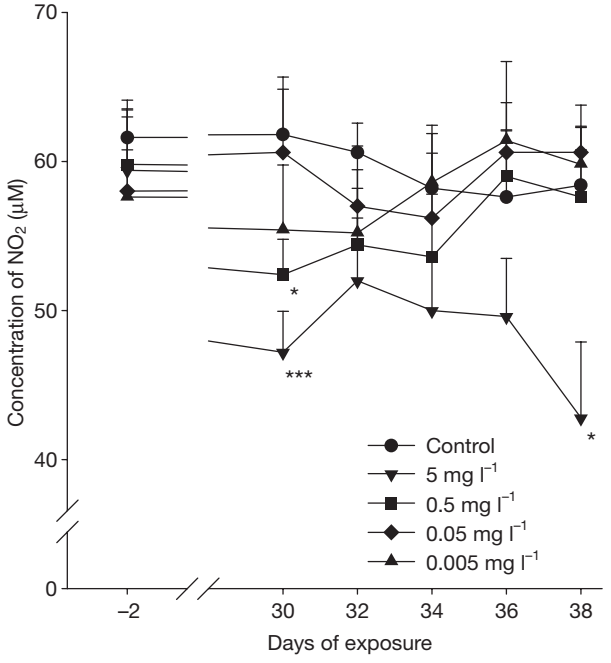

Fig. 4. *Oreochromis mossambicus*. Effect of chronic exposure to Cr (VI) on reactive nitrogen intermediates (RNI) in terms of NO_2 secretion by peripheral blood leucocytes. Points represent mean + SE; n = 5 group^{-1}; *$p < 0.05$; **$p < 0.02$; ***$p < 0.01$

DISCUSSION

The present study clearly shows that chronic exposure to sublethal concentrations of Cr (VI) has an immunomodulatory effect on the immune response of *Oreochromis mossambicus.*

The results of experiments on antibody response indicated that chronic exposure to 0.5 or 5 mg l^{-1} (1 or 10% LC_{50}) of Cr (VI) significantly suppressed antibody titres against heat killed *Aeromonas hydrophila* (Fig. 1). Similar findings have been reported on the reduction of hemagglutination titers against SRBC in the fresh water catfish *Saccobranchus fossilis* exposed to 0.1 to 3.2 mg l^{-1} of Cr (VI) for 28 d (Khangarot et al. 1999). Sugatt (1980) demonstrated that juvenile coho salmon *Oncorhynchus kisutch* exposed to Cr (VI) for 14 d had suppressed agglutinating antibody titers against *Vibrio anguillarum.* Arunkumar et al. (2000) demonstrated the suppression of antibody response to bovine serum albumin (BSA) in *Oreochromis mossambicus* after intraperitoneal injection of Cr (VI). It has been suggested that the suppressive effect might be due to the interaction of chromium with lymphocyte

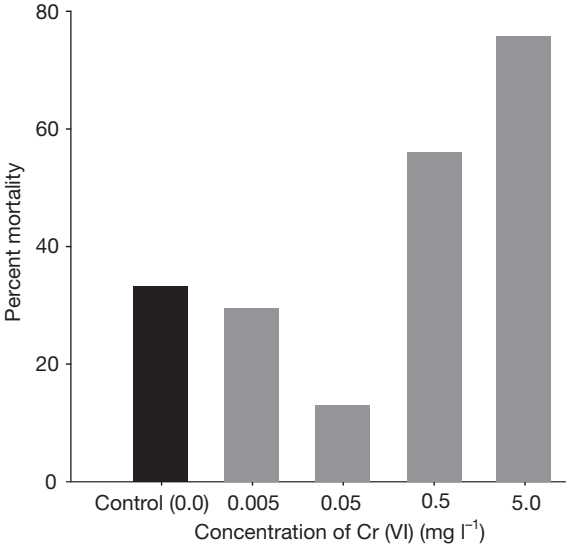

Fig. 5. *Oreochromis mossambicus*. Effect of chronic exposure to Cr (VI) on disease resistance to live virulent *Aeromonas hydrophila*, expressed as percent mortality after 96 h; n = 30 group^{-1}

cell surface proteins, which thereby alters the response of those cells to various stimuli (Snyder & Valle 1991). Further, chromium has been shown to react with cell surface receptors for mitogen, block lymphocyte proliferation, and inhibit immunoglobulin production (Koller 1980, Borella et al. 1990).

In contrast to the above-mentioned observations, chronic exposure to a lower concentration of 0.05 mg l^{-1} (0.1% LC_{50}) of Cr (VI) significantly enhanced the antibody titre to heat killed *Aeromonas hydrophila* (Fig. 1). Viale & Calamari (1984) observed that concentrations of Cr (VI) ranging from 0.05 to 0.2 mg l^{-1} had no effect on the humoral immune response of rainbow trout. Our study provides the first report on immunostimulatory effect of prolonged exposure to a lower concentration of Cr (VI) (0.05 mg l^{-1}) on the antibody response in fish. However, this observation is in agreement with studies on mammalian animal models. Moonsie-Shageer & Mowat (1993) reported enhanced primary immune response to human red blood cells (HRBCs) in stressed feeder calves fed diets supplemented with 0.2, 0.5 or 1.0 mg l^{-1} of Cr (VI). An enhancement of anti-ovalbumin antibody response has also been observed in stressed feeder calves fed with chromium-supplemented feed (Burton et al. 1993). These authors also reported that a diet supplemented with chelated chromium improved antibody titers against infectious bovine rhinotracheitis virus after vaccination.

Lymphocytes producing specific antibodies in spleen of fish can be identified and enumerated by PFC assay. The experiment on the effect of Cr (VI) on antibody producing cells indicated that the number of PFC decreased following chronic exposure to concentrations of 0.5 and 5 mg l^{-1} (Fig. 2). Similar suppressive effects on PFC have been observed in *Saccobranchus fossilis* following exposure to sublethal concentrations of Cr (VI) ranging from 0.1 to 3.2 mg l^{-1} for 28 d (Khangarot et al. 1999). These authors also reported that *in vitro* exposure to Cr (VI) inhibited mitogen induced T-lymphocyte proliferation in spleen and pronephros of *S. fossilis*. It has been suggested that Cr (VI) ions might react with fish splenic and pronephric cell surface proteins, thereby blocking the responses of cells to various stimuli and also inhibiting lymphocyte proliferation (Khangarot et al. 1999).

Here again, chronic exposure to 0.05 mg l^{-1} of Cr (VI) significantly increased the number of PFC (Fig. 2). Earlier studies show that splenocytes isolated from rats exposed to chromium in drinking water exhibited enhanced proliferative response to T- and B-lymphocyte mitogens (Snyder & Valle 1991). Kegley et al. (1996) reported that supplementary Cr in the form of Cr-nicotinic acid enhanced the blastogenic response of feeder calves to phytohemagglutinin (PHA)-stimulated lymphocytes, and also that concanavalin A-induced lymphocyte proliferation was enhanced in chromium (Cr-yeast) supplement-fed cows (Burton et al. 1993). Similarly, van Heugten & Spears (1997) reported increased pokeweed mitogen (PWM) stimulated lymphocyte blastogenesis in pigs fed Cr (VI) or Cr (III) picolinate-supplemented feed. From the above reports, it is evident that trace levels of chromium can enhance the mitogen-induced proliferation of T- and B-lymphocytes. Chang et al. (1996) reported that dietary supplementation of chromium enhanced lymphocyte proliferation in dairy cows, and that this might be associated with chromium and insulin interactions.

Snyder & Valle (1991) reported that Cr (VI) stimulated spontaneous thymidine uptake by rat lymphocytes at concentrations of 0.1 mg l^{-1}, but inhibited uptake at concentrations greater than 0.1 and up to 100 mg l^{-1}. They also reported that T- and B-lymphocyte counts decreased in Wistar rats supplied with 200 mg l^{-1} Cr (VI) in their drinking water, whereas counts increased in rats supplied 100 mg l^{-1} Cr (VI). Thus, our study clearly demonstrated and confirmed that the immunosuppressive or stimulatory effect of Cr (VI) is dependent on concentration.

Serum lysozyme activity was used as a parameter to assess the influence of chronic exposure to Cr (VI) on the humoral component of the nonspecific defense mechanism of *Oreochromis mossambicus*. Sanchez-Dardon et al. (1999) reported that heavy metal pollution affects lysozyme levels and causes alterations of immunoregulatory functions in fish. Our study revealed that chronic exposure to 0.5 or 5 mg l^{-1} of Cr (VI) suppressed serum lysozyme activity. In contrast, chronic exposure to 0.05 mg l^{-1} of Cr (VI) enhanced serum lysozyme activity (Fig. 3). This substantiates the observations of Gatta et al. (2001) that a low level of dietary organic chromium supplementation enhanced the serum lysozyme level in rainbow trout. They reported that both the duration of feeding and the level of supplemented chromium influenced the modulation of lysozyme level. Our study indicated that both positive and negative influences of Cr (VI) on serum lysozyme activity exist.

Campos-Perez et al. (2000) reported that secretion of NO by activated macrophages of rainbow trout inhibits bacterial infection. Results from our study on the effect of chromium on RNI in terms of NO production revealed that chronic exposure to 5 and 0.5 mg l^{-1} of Cr (VI) suppressed NO production (Fig. 4). Steinhagen et al. (2004) reported a similar suppression of NO secretion during *in vitro* exposure of head kidney leucocytes of carp *Cyprinus carpio* to 20 µmol l^{-1} Cr (VI) over 96 h. Tian & Lawrence (1996) reported that chromium suppressed the activity of an NO-producing enzyme (inducible NO synthase) in murine macrophages. Our study revealed negative modulation of RNI production in response to elevated levels of Cr (VI).

Disease resistance was used to assess the overall integrated or combined specific and nonspecific immune mechanisms. Our study demonstrated that chronic exposure to 0.5 or 5 mg l^{-1} of Cr (VI) decreased the disease resistance to *Aeromonas hydrophila* infection (Fig. 5). The percentage mortality of fish increased in a dose-dependent manner from concentrations of 0.5 mg l^{-1} to 5 mg l^{-1}. Several studies demonstrated an increased mortality of fish after viral or bacterial administration following chromium exposure. Khangarot et al. (1999) reported that *Saccobranchus fossilis* exposed to 1.0 or 3.2 mg l^{-1} Cr (VI) for 28 d and administered with live *Aeromonas hydrophila* exhibited higher mortality than control fish. A similar increase in mortality was reported after exposure of coho salmon *Oncorhynchus kisutch* to 0.5 mg l^{-1} Cr (VI) for 14 d and challenged with live *Vibrio anguillarum* (Sugatt 1980). This increase in fish mortality observed in the present investigation was probably due to suppression of nonspecific immune mechanisms.

Interestingly, in our investigation, chronic exposure to 0.05 mg l^{-1} (0.1% LC_{50}) of Cr (VI) increased the disease resistance to *Aeromonas hydrophila* when compared to that of the unexposed control group. Kegley et al. (1996) reported that dietary supplementation of chromium nicotinic acid complex or chromium chloride in feeder calves subjected to physical stress increased the disease resistance against infectious bovine rhinotracheitis virus in young calves. There are no earlier literature reports on chromium treatment increasing disease resistance against bacterial infection in fish. Thus, ours is the first report that prolonged exposure to Cr (VI) has an hormesis-like stimulation of the immune response in fish, i.e. that it is beneficial at lower concentrations and detrimental at higher concentrations.

The most salient conclusion that can be drawn from our study is that, depending on concentration, Cr (VI) modulates the immune response in either a suppressive or a stimulatory manner as shown by its action on lymphocytes, lysozyme, and phagocytic killing mechanisms. These findings may be important when monitoring fish health and risk assessment during periods of fluctuating chromium concentrations in both natural and farm environments.

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