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ARTICLES

Correlation of Transforming Growth Factor-β Messenger RNA (TGF-β mRNA) Expression with Cellular Immunoassays in Triamcinolone-Treated Captive Hybrid Striped Bass

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Abstract.—Assessing fish immune status with molecular markers has been hampered by a lack of specific reagents. A quantitative polymerase chain reaction (PCR) method (reverse transcription quantitative–competitive PCR, RT-qcPCR) for measuring transforming growth factor-β (TGF-β) transcription from a broad range of teleost fish has recently been developed. The quantitative PCR now permits monitoring production of this important immunosuppressive cytokine in response to immunomodulating agents and conditions. We examined anterior kidney and spleen mononuclear cells from hybrid striped bass (female striped bass *Morone saxatilis* × male white bass *M. chrysops*) for production of TGF-β messenger RNA (mRNA) in response to administration of the synthetic glucocorticoid triamcinolone. We also compared TGF-β transcription with anterior kidney macrophage bactericidal activity and splenic lymphocyte blastogenesis. Anterior kidney mononuclear cell TGF-β mRNA levels decreased, whereas bactericidal activity increased. Spleen TGF-β mRNA levels did not change significantly, and splenic lymphocyte pokeweed mitogen stimulation index increased in triamcinolone-treated fish. Since triamcinolone is used therapeutically as a suppressive immunomodulator, the enhanced immune functions indicated by the cellular immunoassays were unexpected; however, the inverse response of TGF-β production and macrophage bactericidal activity was consistent with the known relationship between TGF-β and macrophage activation in mammals. Induced immunomodulation in hybrid striped bass was detectable by both traditional cellular immunoassays and the new RT-qcPCR for TGF-β.

Transforming growth factor-β (TGF-β) is a cytokine with diverse functions affecting cell growth and differentiation, extracellular matrix regulation, wound healing, and immune function (Ruscetti and Palladino 1991; Sasaki et al. 1992; Derynck 1994; McCartney-Francis and Wahl 1994). The immunoregulatory properties of TGF-β are primarily suppressive. Immune functions downregulated by TGF-β include T- and B-cell proliferation, macrophage activation, macrophage respiratory burst activity, and others (Ruscetti and Palladino 1991). Increased levels of TGF-β have been found in several disease states associated with immunosuppression, including different forms of malignancy, chronic degenerative diseases, and AIDS (Ruscetti and Palladino 1991; Sasaki et al. 1992). The ability to measure differences in TGF-β production in fish may provide a valuable tool for evaluating the impact of environmental contaminants, aquaculture management practices, vaccines, and infectious agents on fish immunity.

Evidence for the conserved nature of TGF-β activity in fish is provided by both biological and antigenic cross-reactivity. Bovine TGF-β_1 enhanced respiratory burst activity of resting rainbow trout macrophages at low doses. At higher

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doses, however, bovine TGF-β_1 inhibited activated rainbow trout macrophages and countered the effects of activating signals on resting macrophages (Jang et al. 1994). Chicken antiporcine TGF-β_1 enhanced the ability of supernatants derived from leukocytes of rainbow trout *Oncorhynchus mykiss* to stimulate respiratory burst activity of anterior kidney macrophages (Jang et al. 1995). The TGF-β sequences for rainbow trout (Genbank accession AJ007836; Hardie et al. 1998) and hybrid striped bass (female striped bass *Morone saxatilis* × male white bass *M. chrysops*; Genbank accession AF140363; Harms et al., in press) related to Xenopus TGF-β_5, chicken TGF-β_4, and mammalian TGF-β_1, were recently published.

Based on the hybrid striped bass and rainbow trout sequences, it was possible to design consensus primers that specifically amplify TGF-β mRNA from a broad range of teleost fish species by reverse transcription–polymerase chain reaction (RT-PCR; Harms et al., in press). Adapting a quantitative PCR technique, reverse transcription quantitative–competitive PCR (RT-qcPCR), to teleost fish TGF-β (Harms et al., in press) further allows the investigation of TGF-β production in response to known and suspected immunomodulating agents or conditions.

Glucocorticoids are primarily considered suppressive immunomodulating agents and are used therapeutically for their anti-inflammatory and immunosuppressive properties (Plumb 1999), although physiologic immune enhancing functions for glucocorticoids have recently been reviewed (Wilckens and De Rijk 1997). The mammalian TGF-β_1 promoter region contains a glucocorticoid response element, and human T-cells (but not glial cells) up-regulate TGF-β_1 mRNA and protein production in response to the synthetic glucocorticoid dexamethasone (Batuman et al. 1991, 1995). Triamcinolone acetonide is a long-acting synthetic glucocorticoid with five times the relative anti-inflammatory potency as cortisol and with minimal mineralocorticoid activity (Plumb 1999). It is dosed at 0.11–0.22 mg/kg intramascularly or subcutaneously for therapeutic effects in dogs (Plumb 1999). Experimentally it has been used at 200 μg/g (= mg/kg) to immunosuppress common carp *Cyprinus carpio* in an *Ichthyophthirius multifiliis* challenge study (Houghton and Matthews 1986), at 20 mg/kg to immunosuppress rainbow trout in response to a *Yersinia ruckeri* bacterin (Anderson et al. 1982), and at 100 mg/kg to immunosuppress striped bass in an infectious pancreatic necrosis virus challenge study (Wechsler et al. 1986).

Our objectives were to determine the in vivo effects of a known immunomodulator, triamcinolone, on the transcription levels of TGF-β in hybrid striped bass and to compare TGF-β mRNA production with macrophage and lymphocyte function assays.

Methods

Fish.—Fourteen adult hybrid striped bass were purchased from a commercial aquaculture facility and transported to North Carolina State University's (NCSU's) College of Veterinary Medicine. Fish were treated prophylactically for external parasites with a 3-min dip in 25 g salt/L plus 0.5 mL formalin/L. Two fish died shortly after transport. The remaining 12 were kept for 7 months in a 1,000-L flow-through freshwater tank under Institutional Animal Care and Use Committee approval. Water conditions were 18°C, pH 6.8–7.2, ammonia less than 0.1 mg/L, and nitrite less than 0.1 mg/L. Salinity was held initially at 3 g/L and gradually reduced to 0 g/L over 14 d. Fish were fed commercial trout pellets and kept on a photoperiod of 14 h light : 10 h dark. No external parasites were detected by microscopic examination of gill biopsies and skin scrapes at the time of entry into the NCSU facility or at the time of sampling. No external or internal gross lesions were noted. One fish died shortly before sampling due to spinal trauma following a collision with the tank wall at feeding time.

Hybrid striped bass were alternately designated control ($N = 6$) or experimental ($N = 5$) based on capture order at the time of the experiment. All fish were anesthetized with tricaine methanesulfonate (MS-222) at 150 mg/L. Control fish were sampled immediately to minimize effects of endogenous cortisol release resulting from capture and MS-222 anesthesia. They were bled from the caudal vein with heparinized syringes—as much blood as possible was taken to reduce peripheral blood content of spleen and anterior kidney—and then euthanatized by immersion in 250 mg MS-222/L followed by cervical transection. Weight and length were recorded, and spleen and anterior kidneys were harvested aseptically into sterile cell culture medium, complete RPMI (RPMI-1640 plus 10% heat-inactivated fetal bovine serum, 100 U penicillin/mL, 100 μg streptomycin/mL, and 2 mM EDTA). Blood and organs were held on wet ice for transport to the laboratory and processed within 2 h. Experimental fish were injected with 10 mg triamcinolone acetonide/kg (Kenalog-10, Apothecon, Princeton, New Jersey; 10-mg/mL

suspension) intracoelomically, placed in anesthesia-free water to recover, and returned to the original tank. After 3 d experimental fish were processed in the same manner as for control fish.

Sample processing.—Blood was centrifuged at 800 × gravity (*g*) for 20 min and plasma was harvested for determination of electrolytes (Na^+, Cl^-, total CO_2), glucose, and cortisol. Plasma electrolytes were measured on an automated analyzer (Monarch Plus, Instrumentation Laboratory, Lexington, Massachusetts), and osmolality on a μ-Osmette micro-osmometer (Precision Systems, Natick, Massachusetts). Plasma cortisol was determined using an automated fluorescence polarization immunoassay (Abbott Laboratories, North Chicago, Illinois) previously validated for use in hybrid striped bass (Noga et al. 1994). Spleen and anterior kidneys were each split in two for TGF-β RT-qcPCR (both organs), macrophage bactericidal assay (anterior kidney), and lymphocyte blastogenesis assays (spleen).

TGF-β RT-qcPCR.—Mononuclear cells for RT-qcPCR were prepared as previously described (Harms et al., in press). Organs were minced finely, forced through a fine wire mesh, and resuspended in complete RPMI. Organ homogenates were centrifuged on two-step Percoll gradients (specific gravity 1.053 and 1.066 g/mL in 0.15 M saline) at 400 × *g* for 5 min then at 800 × *g* for 20 min at 4°C. Mononuclear cells were harvested from the 1.053–1.066-g/mL interface. Cells were washed twice in complete RPMI. Viable cell counts were performed with cells suspended in 0.2% trypan blue, and differential counts were performed on cytospin preparations stained with LeukoStat (Fisher Scientific, Pittsburgh, Pennsylvania). Cell viability typically exceeded 95%.

Total RNA was isolated by the guanidine thiocyanate method (Tri Reagent, Molecular Research Center, Cincinnati, Ohio). The RNA pellet resuspended in sterile diethyl pyrocarbonate-treated water at a concentration of 5×10^4 cell equivalents/μL. Messenger RNA was reverse transcribed (Superscript II RT, Gibco BRL, Gaithersburg, Maryland) to cDNA with oligo-dT_{15} priming of 3×10^6 cell equivalents of total RNA. Samples of cDNA were stored at −20°C until used. Negative RT controls were run in parallel.

The RT-qcPCR was performed based on a previously described procedure (Rottman et al. 1996) and further developed using primers and competitive fragments specific for teleost fish TGF-β and beta-actin (Harms et al., in press). Each competitive PCR contained 2.5×10^4 cell equivalents of cDNA as prepared above. Final reaction volume of the competitive PCR was 25 μL, reached by adding 20 μL of a PCR master mix to 5 μL of each competitive fragment dilution (1.5×10^8 copies down to 6 copies) in a Thermowell 96-well thin-wall polycarbonate plate (Costar, Acton, Massachusetts) on ice. Final reaction concentrations were 1× PCR buffer (10 mM tris–HCl at pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl), 250 μM each deoxynucleotide triphosphate (dNTP), 0.375 μM each primer, and 30 μU/μL *Taq* DNA polymerase. Reactions were cycled on a PTC-100 thermocycler (MJ Research, Inc., Watertown, Massachusetts) at 94°C for 1 min; then 35 cycles of denaturing at 94°C for 30 s, annealing at 55°C for 1.5 min, and extension at 72°C for 2 min; and a final extension at 72°C for 7 min.

The PCR products were separated in 2% agarose gels in TAE (tris–acetate–EDTA), stained with ethidium bromide, and photographed on a 300-nm ultraviolet transilluminator. The images were digitized and analyzed with the Alphaimager 2000 Documentation and Analysis System (Alpha Innotech Co., San Leandro, California). Fluorescence of target and competitor bands in each lane were measured and expressed as area under the curve. Fluorescence differences due to molecular weight differences were compensated by the formula

$$\text{corrected fluorescence ratio} = \frac{\text{target fluorescence (area)}}{\text{competitor fluorescence (area)}} \times \frac{\text{competitor size (bp)}}{\text{target size (bp)}};$$

bp = base pairs. The log of the corrected fluorescence ratio was plotted against the log of the number of copies of competitor in the sample, and the point of molecular equivalence (the point at which the copy number of target cDNA equals the copy number of competitor DNA) is the *x*-intercept. Finally, to control for sample-to-sample variation in RNA isolation, reverse transcription, amplification, and gel loading during quantification, TGF-β results are normalized to those of the housekeeping gene, beta-actin.

Cellular immunoassays.—Spleen and anterior kidney mononuclear cells for cellular immunoassays were isolated by using a modification of the procedures described by Sharp et al. (1991). Cells were kept cold during processing except where noted. Aseptically harvested anterior kidney tis-

sues from individual fish were homogenized in 10 mL of L-15 medium supplemented with 2% fetal bovine serum (FBS), 100 U penicillin/mL, 100 μg streptomycin/mL, and 10 U sodium heparin/mL (L-15/2%FBS). Single-cell suspensions were produced by repeated expression through an 18-gauge needle from a 3-mL syringe. Spleen tissue was minced to facilitate syringe passage. Tissue fragments were allowed to settle for at least 10 min and the cell suspensions were removed. Washed cells were resuspended in 6 mL L-15/2%FBS and layered on Percoll (specific gravity, 1.047 g/mL) in Hanks' balanced salt solution (HBSS) without phenol red. Cell suspensions on Percoll were centrifuged at 4°C for 20 min at 400 × *g*, and then mononuclear cells were removed from the medium–Percoll interface. Cells were washed and viable cell counts were determined by trypan blue exclusion (0.1% trypan blue in L-15/2%FBS). To enrich for adherent cells, anterior kidney mononuclear cells were resuspended at 2×10^7 viable cells/mL in L-15 supplemented with 0.1% FBS, 100 U penicillin/mL, and 100 μg streptomycin/mL (L-15/0.1%). These cells were loaded in 96-well tissue culture plates at 100 μL/well and incubated for 2 h in a humidified container at 20°C. After incubation, the L-15/0.1% containing nonadherent cells (macrophage plates) was removed and replaced by 100 μL (per well) of L-15 supplemented with 5% FBS, 100 U penicillin/mL, and 100 μg streptomycin/mL (L-15/5%) at 20°C. Adherent cells were evaluated using the macrophage bactericidal assay described below. Splenic mononuclear cells were resuspended at 2×10^7 viable cells/mL in L-15/5%. These cells were loaded in 96-well tissue culture plates at 50 μL/well and then further treated for mitogenesis evaluation as described below.

Bactericidal assay (anterior kidney adherent cells).—Bactericidal activity was determined by using a modification of the assay described by Graham et al. (1988). The temperatures of all solutions were adjusted to 20°C before use. Forty-eight-hour cultures of *Yersinia ruckeri* (Hagerman strain; National Fish Health Research Laboratory isolate 11.40) were washed three times in HBSS, and the suspension density was adjusted to 0.15 optical density at 600 nm (OD_{600}) in HBSS.

After 36 h of incubation in a humidified container at 20°C, the L-15/5% was removed from the wells of macrophage plates and replaced with 100 μL of unsupplemented L-15/well. The L-15 was then replaced with an equal volume of L-15/5% without antibiotics, and *Y. ruckeri* suspension was added at 25 μL/well. Plates were incubated for 4 h at 20°C in a humidified container. After incubation, medium was removed from the wells and replaced with 50 μL (per well) of 0.2% Tween 20 in sterile, distilled, deionized water. The resulting well contents were serially diluted (log 10) in tryptic soy broth. Dilutions were plated as 10-μL drops on tryptic soy agar plates and incubated 24 h at room temperature. The number of colony-forming units (CFUs) was determined for each well at the 10^{-4} or 10^{-5} dilution. Bactericidal activity was expressed as

$$\text{percent CFU reduction} = (1 - \text{CFU treated/CFU control}) \times 100.$$

The ''CFU treated'' was the mean number (3 replicates) of CFUs in wells containing adherent leukocytes, and ''CFU control'' was the mean number (6 replicates) of CFUs from wells with medium only; CFU treated was replicated on a per-fish (cell source) basis and CFU control was replicated on a per-plate basis (i.e., all the CFU treated mean values from a given plate were compared with a single CFU control mean value).

Mitogenesis assays (splenic mononuclear cells).—Mitogenesis assays were performed, following a modification of the procedure described by Daly et al. (1995), by using doses and kinetics optimized for hybrid striped bass (Wang et al. 1997). Either concanavalin A (Con A) at 10 μg/mL, pokeweed mitogen (PWM) at 50 μg/mL in L-15/5%, or L-15/5% only was added at a volume of 50 μL/well immediately after leukocyte plating. All Con A-treated cells with negative controls were conducted in a separate plate from the remaining mitogen and negative-control groups. Incubation times were 60 h for the Con A plate and 108 h for the PWM plate. Incubation occurred in a humidified container at 20°C. After the primary incubation, 10 μL of a 5-mg/mL solution of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) in L-15 was added to each well and the plates were incubated for an additional 4 h as above. After incubation, medium was removed from the wells and replaced by 200 μL of DMSO and 25μL of glycine buffer (100 mM glycine, 100 mM sodium chloride, pH = 10.5) per well. The contents of the wells were mixed by pipetting, and the OD_{650} of the well contents was determined by using a microplate reader. All treatments were performed in quadruplicate. The value obtained for each treatment was taken to be the mean of the replicates. The results of the assays were expressed

TABLE 1.—Lymphoid organ immunologic measures and plasma chemistries for control ($N = 6$) and triamcinolone-treated ($N = 5$) hybrid striped bass compared by Wilcoxon rank-sum tests; ns = not significant. Values for variables in each treatment are medians (lower quartile, upper quartile).

Variable	Treatment		P
	Control	Triamcinolone	
TGF-β:beta-actin[a]			
Anterior kidney	0.0039 (0.0030, 0.0056)	0.0024 (0.0022, 0.0033)	0.028
Spleen	0.011 (0.0047, 0.015)	0.0068 (0.0055, 0.011)	ns
Anterior kidney bactericidal activity (% CFU[b] reduction)	44.9 (24.5, 61.2)	71.5 (66.1, 75.1)	0.011
Stimulation index			
Spleen PWM[c]	−0.025 (−0.095,−0.0075)	0.19 (0.095, 0.20)	0.0061
Spleen Con A[d]	0.10 (0.058, 0.17)	0.090 (0.020, 0.20)	ns
Viable cell recovery			
Anterior kidney (number of cells)	4×10^8 (3×10^8, 5×10^8)	2×10^8 (1×10^8, 3×10^8)	0.0062
Spleen (number of cells)	7.7×10^7 (6.2×10^7, 1×10^8)	3.7×10^7 (3.2×10^7, 5.6×10^7)	0.018
Na^+ (mmol/L)	176 (174, 178)	167 (162, 169)	0.0061
Cl^- (mmol/L)	140 (135, 141)	128 (116, 130)	0.0056
Total CO_2 (mmol/L)	6 (5, 7.8)	8 (6.5, 10.5)	ns
Osmolality (mOsm/kg)	358 (353, 366)	342 (334, 353)	0.0219
Glucose (mg/dL)	102 (95, 108)	130 (124, 148)	0.0062
Cortisol (μg/dL)	10.5 (8.0, 20.2)	0.4 (0.02, 0.8)	0.0062

[a] Ratio of transforming growth factor-β to beta-actin.
[b] Colony-forming units.
[c] Pokeweed mitogen.
[d] Concanavalin A.

as a stimulation index (SI) value (Daly et al. 1995):

$$\text{SI} = \frac{\text{replicate mean OD}_{650}\text{ mitogen}}{\text{replicate mean OD}_{650}\text{ TCM}} - 1.$$

Stimulation values were expressed on a per-fish (cell source) basis.

Statistical analyses.—Statistical analysis was performed with a commercial statistical package (JMP 3, SAS Institute, Inc., Cary, North Carolina). Because of the nonnormal distribution of some data (Shapiro–Wilk *W*-test), nonparametric statistical methods were employed throughout. Experimental and control groups were compared by the Wilcoxon rank-sum test. Ratios of TGF-β: beta-actin and cellular immunoassays were examined for correlation by Kendall's tau-b. Significance was set at $P < 0.05$.

Results

Control fish weighed 712–1,450 g and were 33–39 cm long (four males and two females). Experimental fish weighed 734–1,257 g and were 34–39 cm long (three males and two females).

Production of TGF-β mRNA, anterior kidney macrophage bactericidal activity, splenic mononuclear cell mitogen stimulation indices, total viable cell recoveries from anterior kidney and spleen, plasma chemistries, and plasma cortisol are shown in Table 1. Significant decreases were observed in triamcinolone-treated hybrid striped bass for TGF-β mRNA production (Figure 1), total cell recovery from both anterior kidney and spleen, and plasma Na^+, Cl^-, osmolality, and cortisol. Significant increases were noted for anterior kidney macrophage bactericidal activity (Figure 2), splenic mononuclear cell PWM stimulation index, and

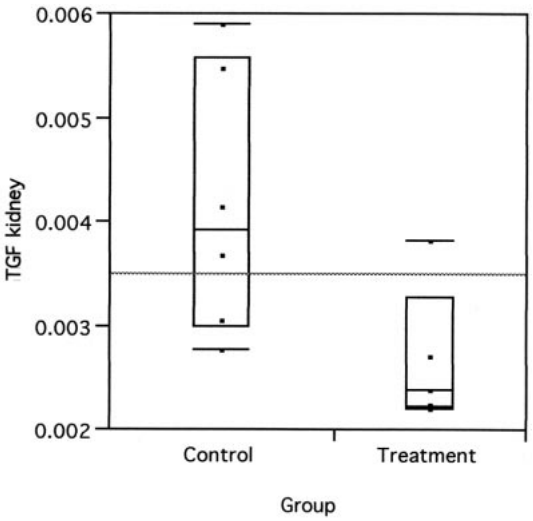

FIGURE 1.—Ratios of TGF-β: beta-actin in anterior kidney mononuclear cells from control ($N = 6$) and triamcinolone-treated ($N = 5$) hybrid striped bass (Wilcoxon rank-sum test, $P = 0.028$). Quantile boxes show the 10th, 25th, 50th (median), 75th, and 90th quantiles; dots indicate individual data points; horizontal line indicates the total response sample mean.

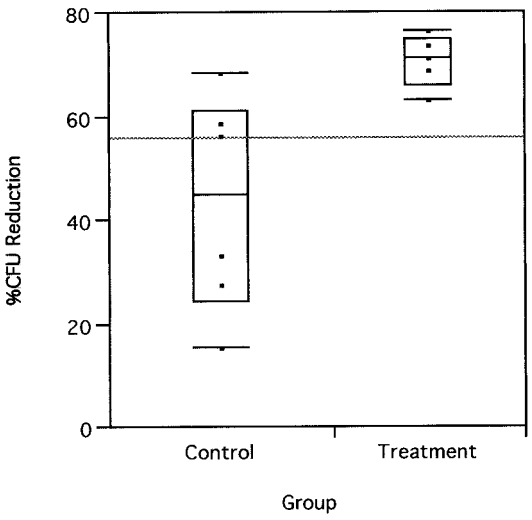

FIGURE 2.—Anterior kidney macrophage bactericidal activity expressed as percent reduction of colony-forming units (CFU) in control ($N = 6$) and triamcinolone-treated ($N = 5$) hybrid striped bass (Wilcoxon rank-sum test, $P = 0.011$). Quantile boxes show the 10th, 25th, 50th (median), 75th, and 90th quantiles; dots indicate individual data points; horizontal line indicates the total response sample mean.

plasma glucose. Anterior kidney TGF-β: beta-actin ratios and bactericidal activity were inversely correlated (Kendall's tau-b $= -0.7818$, $P = 0.0008$). No other immunological measures correlated significantly.

Although total numbers of viable cells isolated from both spleen and anterior kidney declined significantly (Table 1), based on differential counts of cytospin slides from the two-step Percoll gradients, mononuclear cell percentages did not differ between treatment and control groups (median [lower, upper quartiles]: splenic lymphocytes, control 51.0% [45.8%, 64.5%] versus treated 52.0% [42.5%, 54.5%]; splenic macrophages, control 22.0% [15.8%, 27.0%] versus treated 23.0% [16.5%, 28.0%]; anterior kidney lymphocytes, control 42.0% [31.0%, 59.5%] versus treated 68% [34.5%, 70.5%]; anterior kidney macrophages, control 30.5% [16.0%, 39.0%] versus treated 15.0% [9.0%, 32.0%]; Wilcoxon rank-sum tests, $P \gg 0.05$ for all comparisons.) There were variations in minor cell types (splenic erythrocytes, control 1.0% [0.8%, 1.8%] versus treated 7.0% [3.0%, 1.5%]; splenic blast cells, control 16.0% [10.5%, 21.0%] versus treated 5.0% [3.5%, 7.0%]; anterior kidney blast cells, control 16.0% [10.5%, 21.0%] versus treated 3.0% [1.0%, 5.5%]; Wilcoxon rank-sum tests, $P < 0.05$; anterior kidney erythrocytes, control 2.5% [1.5%, 4%] versus treated 1.0% [0.0%, 1.5%], $P > 0.05$) that do not correlate with TGF-β mRNA production (Harms et al., in press).

Discussion

The observed decrease in plasma cortisol indicates nearly complete suppression of the hypothalamic–hypophyseal–interrenal axis by triamcinolone administration. Plasma electrolyte alterations, reduced osmolality, and increased glucose are consistent with endogenous stress-induced increases in cortisol in fish (Harms et al. 1996; Reubush and Heath 1997).

Increased anterior kidney macrophage bactericidal activity and splenic mononuclear cell PWM stimulation index, and decreased anterior kidney mononuclear cell TGF-β mRNA production were unexpected in light of known immunosuppressive effects of glucocorticoids (Plumb 1999) and the presence of a glucocorticoid response element in the regulatory region of mammalian TGF-β_1 (Batuman et al. 1991, 1995). However, the immunologic changes were consistent with each other and with the expected inverse relationship between TGF-β production and macrophage activation (Ruscetti and Palladino 1991). Although glucocorticoids are known for their immunosuppressive

effects (Plumb 1999), and increased endogenous cortisol is a key component of the stress response in fish (Noga et al. 1994; Schreck 1996), certain acute stressors can enhance some immunologic responses. Evidence that glucocorticoids can direct and enhance some immune functions in mammalian systems has been recently reviewed (Wilckens and De Rijk 1997). Examples from fish immunology include increased nonspecific cytotoxic cell activity in tilapia *Oreochromis* sp. after temperature stress (Jaso-Friedmann et al., in press) and decreased apoptosis of peripheral leukocytes of channel catfish *Ictalurus punctatus* after net confinement (Alford et al. 1994). Chinook salmon *Oncorhynchus tshawytscha* subjected to varied acute stressors (e.g., confinement, transport) had increased plasma cortisol, reduced anterior-kidney-specific antibody-producing cells, and reduced resistance to *Vibrio anguillarum* 4 h after stress. Yet at 24 h, antibody-producing cells and plasma cortisol were unchanged compared with controls, but resistance to *V. anguillarum* was enhanced (Maule et al. 1988). And juvenile common carp *Cyprinus carpio* experienced increased secondary antibody responses to human gamma globulin or trinitrophenylated sheep red blood cells when cortisol was administered simultaneously with primary antigen injection or 3 d before and after the injection (Ruglys 1985).

It is possible that a different time course or triamcinolone dose would have yielded different results in hybrid striped bass than those reported here. The triamcinolone dose used in the present study (10 mg/kg) is high relative to therapeutic doses in mammals (0.11–0.22 mg/kg; Plumb 1999), comparable with a dose reported to reduce splenic antibody-producing cells and antibody titers in response to *Y. ruckeri* bacterin in rainbow trout (20 mg/kg; Anderson et al. 1982), but low compared with reported doses that reduced carp resistance to ichthyophthiriasis (200 mg/kg; Houghton and Matthews 1986) and reduced striped bass antibody response to and viremia clearance of infectious pancreatic necrosis virus (IPNV, 100 mg/kg; Wechsler et al. 1986). The triamcinolone dose can also be compared with other glucocorticoid doses used in fish based on the relative anti-inflammatory potency of triamcinolone being five times that of hydrocortisone (Plumb 1999). A dose of hydrocortisone at 1 mg/kg (equivalent triamcinolone dose, 0.2 mg/kg) reduced circulating Ig^+ cells and mitogenic response of peripheral blood cells to lipopolysaccharide for 2–3 d after intraperitoneal injection in Atlantic salmon *Salmo salar* (Espelid et al. 1996), and 50 mg hydrocortisone/kg (equivalent triamcinolone dose, 10 mg/kg) administered in a slow-release formulation (maximum release 17 d postimplantation) reduced plaque-forming cell response in winter flounder *Pleuronectes americanus* (Carlson et al. 1993). Thus, the dose of 10 mg triamcinolone/kg used in the present study sits within a 3-log range of glucocorticoid doses demonstrated to have immunological effects in fish. Sampling for TGF-β mRNA production in response to multiple glucocorticoid dosages and at multiple times would be beneficial.

A possible interpretation for the unexpected decline in TGF-β transcription and increase in macrophage bactericidal activity is that triamcinolone might have preferentially induced down-regulation, apoptosis, or redistribution of anterior kidney suppressor cells (possibly TGF-β-producing lymphocytes), releasing macrophages from their regulatory influence (Zapata et al. 1992). Total viable cells (predominantly mononuclear cells) isolated from anterior kidney and spleen of triamcinolone-treated fish were approximately half that of untreated controls (Table 1), indicating a dramatic effect on cells of the lymphoid system. Lymphocyte and monocyte/macrophage percentages from anterior kidney and splenic mononuclear cells did not differ between treated and control fish based on microscopic examination of cytospin slides, but that does not rule out the possibility of differential effects on lymphocyte subtypes. The varied responses to two mitogens (increased response to PWM, no change in response to Con A) by splenic lymphocytes from treated versus untreated fish suggest that triamcinolone affected a heterogeneous lymphocyte population differentially. Additional cellular partitioning could reveal further information about glucocorticoid-induced immunomodulation in fish.

Unlike many other cytokines for which production is primarily transcriptionally regulated, TGF-β production and activity are regulated at both transcriptional and posttranscriptional steps (Roberts and Sporn 1996). The TGFs-β are made as larger precursors, consisting of a short *N*-terminal signal peptide, a long pro-segment (latency-associated polypeptide, LAP), and a C-terminal peptide that is cleaved to form the mature TGF-β monomer. The active form is a disulfide-linked homodimer of the mature monomer. The TGF-β is normally secreted as a complex of the mature homodimer linked noncovalently with two LAPs. Therefore, observations of altered transcription levels may be loosely tied to changes in immune

functions. The ability to detect and measure active TGF-β protein in fish would improve assessment of TGF-β responses and effects, but reagents specific to different TGF-β isoforms from varied species of fish are currently not available. The RT-qcPCR technique used in this study permits monitoring production of a specific TGF-β isoform at the transcriptional level in multiple species of fish (Harms et al., in press).

In conclusion, we have demonstrated altered transcription of TGF-β by anterior kidney mononuclear cells in response to administration of an immunomodulating agent. The observed decline in TGF-β transcription was inversely correlated with anterior kidney macrophage bactericidal activity. The RT-qcPCR for measuring TGF-β transcription thus shows promise as a useful adjunct technique for assessing immune status of teleost fish, as part of a suite with other established immunoassays, and for investigating molecular mechanisms of teleost fish immune responses.

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