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Developmental and Comparative Immunology 30 (2006) 817–830
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

Immunomodulatory effects of β-glucan on neutrophil function in fathead minnows (*Pimephales promelas* Rafinesque, 1820)

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Received 24 April 2005; received in revised form 1 November 2005; accepted 22 November 2005
Available online 21 December 2005

Abstract

Stimulatory effects of yeast β-1,3–1,6-glucans on neutrophils have long been recognized, but effects of glucans on degranulation of primary granules in fish neutrophils have not been previously reported. Neutrophil function was monitored during in vitro and in vivo application of glucans to non- (NS), acute- (AS) and chronically stressed (CS) fish. β-Glucan proved to be a strong and quick (80%, 2 min) stimulant of degranulation. Dietary glucan increased degranulation in NS fish, and prevented a decrease in AS fish. Degranulation in CS fish returned to NS levels 3 days after the glucan diet was fed. Fathead minnows appear to be a useful model to investigate neutrophil degranulation in fish exposed to different environmental conditions and immunomodulators. Use of β-glucans in fish diets prior to AS and during chronic stress can enhance neutrophil function, potentially increasing disease resistance and survival rates after transportation or exposure to poor water quality.

Keywords: β-Glucan; Neutrophil function; Stress; Fish; Degranulation

1. Introduction

β-1,3–1,6-Glucans are complex polysaccharide components of cell walls found in a large variety of organisms [1]. Stimulatory effects of β-1,3–1,6-glucans on neutrophils, as well as other components of the immune system, have long been recognized [2,3]. Glucan-specific receptors are present on phagocytic cell membranes of several species, including fish neutrophils [4,5], and potent activation of neutrophil function, including

Abbreviations: TAN, total ammonia nitrogen; TNN, total nitrite nitrogen; CaI, calcium ionophore; $HBSS^{CMF}$, Hank's balanced salt solution without Ca^{2+} and Mg^{2+}; PMA, phorbol myristate acetate; GB, β-glucan from barley; GY, β-glucan from yeast Sigma; MG, MacroGard Feed ingredient; soluble MG, MacroGard AquaSol; Z, zymosan; Cyt *C*, cytochrome *C*; MPO, myeloperoxidase; CTAB, cetyltrimethylammonium bromide; PMSF, phenylmethylsulfonyl fluoride; TMB, tetramethylbenzidine hydrochloride

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doi:10.1016/j.dci.2005.11.004

an increase in phagocytosis and killing, has been described in vitro [6,7]. The stimulatory effects of dietary β-glucan from baker's yeast (*Saccharomyces cerevisiae*) on neutrophil function, and increased disease resistance, have been recently demonstrated in several crustacean, fish and amphibian species [8–10].

Neutrophils are an important component of host defense against many bacterial, viral and fungal infections, and the evaluation of neutrophil function is valuable for assessment of the health status of individuals and animal populations [11]. Fish neutrophils have phagocytic, chemotactic and bactericidal functions, an intense respiratory burst, and peroxidase (myeloperoxidase, MPO) activity [12–14]. The process of degranulation is essential for the release of MPO and activation of the halide production pathway, as well as release of a diverse cocktail of antimicrobial enzymes. Changes in degranulation activity can influence the killing potential of the neutrophil, and potentially reduce the ability of the organism to defend against infection [15,16]. Measuring exocytosis of MPO from primary neutrophil granules in vitro is a direct, rapid and quantitative method to assess the degranulation process in fish neutrophils [16,17].

Severe or chronic stress is often associated with poor performance and has long been suspected to cause immunosuppression in cultured fish [18]. Effects of acute and chronic aquaculture stress on cortisol concentrations, innate and acquired immune function (cellular and humoral), and disease resistance have been reported [18]. Markedly elevated, as well as chronically increased concentrations of cortisol, act as inhibitors of neutrophil function [19], and a significant decrease in degranulation of fish neutrophil primary granules was observed after handling and crowding stress [20].

If fish are injured or exposed to other harmful conditions, there is a cause for concern not only in terms of responsible stewardship of fish populations, but also in terms of the welfare of individuals [21]. Decrease in neutrophil function can lead to increase in disease occurrence, and individuals with decreased or missing neutrophil degranulation had high incidence of bacterial and fungal infections often leading to terminal outcome [22]. Therefore, stress caused decrease in neutrophil degranulation could lead to reduced disease resistance and increased mortality rates, causing pain and distress in individual fish.

The fathead minnow (*Pimephales promelas*, Rafinesque, 1820) is a viable model to measure neutrophil function, such as degranulation and oxidative burst, during handling and crowding stress, and chemical compound (anesthetics) administration [20]. The widespread use of fathead minnows as laboratory models in toxicology research, their aquacultural and ecological relevance, and availability of functional assays, make them a species of choice for immunological research, including the effects of stress, and dietary immunomodulators on neutrophil function [20,23].

The effect of glucans on degranulation of neutrophil primary granules in fish, and the dietary application of β-glucans from baker's yeast in fathead minnows have not been reported. The purpose of this study was to determine the effects of glucans obtained from different commercial sources (Sigma; Biotec Pharmacon ASA, Norway) and species (baker's yeast, barley), when used as in vitro stimulants in fathead minnow neutrophil functional assays, and the optimal stimulant for dietary immunomodulation. In this study, commercially available particulate β-1,3–1,6-glucan from baker's yeast was found to be a potent stimulator of fish neutrophil degranulation and an optimal immunomodulator of fathead minnow neutrophil function during stress conditions.

2. Materials and methods

2.1. Fish

Adult fathead minnows with an average weight of 3 g were maintained in the Department of Natural Resource Ecology and Management, Iowa State University, Ames, IA, USA. Fish were held in 300–1000 L tank recirculation system supplied with dechlorinated tap water at 20 °C and fed daily with dried flake food (Aquatox®, Ziegler Bros Inc., PA, USA). Fish designated for experiments were fed 5% of the body weight, twice a day with a prepared basal diet at least 3 weeks prior to experiment. Water quality parameters were measured two times per week: water temperature was 20 ± 1 °C, pH was 8.0 ± 0.2, dissolved O_2 was $7 \pm 1\,mg\,L^{-1}$, total ammonia nitrogen (TAN) was $<0.5\,mg\,L^{-1}$ and total nitrite nitrogen (TNN) was below detection limit (HACH spectrophotometer 2000NR). Fathead minnows were cared for in accordance with approved Iowa State University animal care guidelines.