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Developmental and Comparative Immunology 30 (2006) 393–406
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

Plasma proteomic analysis of the acute phase response of rainbow trout (*Oncorhynchus mykiss*) to intraperitoneal inflammation and LPS injection

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Received 29 March 2005; accepted 5 June 2005
Available online 6 July 2005

Abstract

Few acute phase proteins are known in fish and better knowledge of them would provide a basis for more reliable methods to objectively assess fish health and welfare. An acute phase response was induced in rainbow trout (*Oncorhynchus mykiss*, Walbaum) by inflammation triggered by intraperitoneal administration of purified *Aeromonas salmonicida* lipopolysaccharide emulsified in Freund's incomplete adjuvant (LPS/FIA) or a commercial oil-based multivalent vaccine. Acute phase proteins were characterized by comparative densitometry of plasma proteins separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and identified by MALDI-TOF and ESI MS/MS mass spectrometry. In one experiment, plasma samples were compared between treatment and control groups in which fish were terminally bled. In another experiment, individual fish were sampled repeatedly. Proteins scored as increased were those whose normalized value increased three-fold or greater between pre- and post-stimulus. Proteins scored as decreased were those whose normalized values decreased two-fold or greater. Unaltered proteins were those that were not altered or did not meet either of these criteria. Proteins that were absent in pre-stimulus gels but present in post-stimulus profiles were considered to be induced. Only those proteins that were altered in all fish for a given treatment were considered. In both experiments, protein p36 was increased up to 13-fold and several proteins were detected that had not been previously. In all fish treated with LPS/FIA, p9.5 was consistently increased an average of 75-fold in plasma. We have constructed a plasma protein panel of eight increased or induced proteins (p9.5, p10.5, p24a, p24b, p24c, p25a, p36 and p37), one decreased (p16) and two that are unaltered (p28a, p28b) in rainbow trout following inflammation or injection with LPS/FIA. Proteins from this panel that were similar to previously identified proteins were pre-cerebellin-like (p24a), transferrin (p37) and apolipoprotein (p10.5, p24c and p28).

Keywords: Rainbow trout; *Oncorhynchus mykiss*; Acute phase response; Plasma proteins; 2D-PAGE; Proteomics; Inflammation; *Aeromonas salmonicida*; LPS.

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doi:10.1016/j.dci.2005.06.002

1. Introduction

The acute phase response (APR) has been defined as a rapid, orchestrated, physiologically induced response to tissue injury, infection, neoplasia, trauma and stress [1,2]. In homeothermic vertebrates, the APR is marked by systemic and local reactions including the induction of fever, hematopoiesis, lymphoid proliferation, adrenocortiotropic hormone production, hypoferremia, complement activation and differential production of a number of acute phase plasma proteins (APP) secreted by the liver [3]. Many proteins that increase in the plasma (positive APP) result from increased expression and secretion by the liver. Those that decrease as a result of reduced expression rather than consumption or loss are referred to as negative APP [4,5]. Many APPs have various functions in host defense or adaptation to infection. For example, some are consumable proteins required for homeostasis and regulation of the inflammatory response, whereas others are transport proteins. Several have direct anti-microbial properties, for example, neutralization of microbial proteases, restriction of microbial resources, opsonization or microbiocidal activity [6–8].

Homeothermic animals mount a vigorous and rapid APR that can be monitored to gauge the process of inflammatory disease. By contrast, studies to date have suggested that ectothermic vertebrates have a muted APR that is delayed compared to mammals [9,10]. For ectotherms such as teleost fish, low temperatures can be non-permissive for an effective adaptive immune response and they therefore must rely on innate immunity and the APR to provide a more immediate first line of defense [10–12]. For example, major histocompatability receptor expression is down regulated in carp at low water temperatures [13] suggesting an increased dependence on innate immune proteins including proteins secreted by the liver as part of an APR [14]. Teleost fish express many homologs to mammalian APPs, including C-reactive protein (CRP) [15,16], serum amyloid P (SAP) [17], serum amyloid A (SAA) [2], transferrin [18], and various proteins of the complement component pathway including C3, C4 and factor B [19]. Proteins now considered by some to be positive teleost APP are SAA [2], CRP [15,20], transferrin [9,21], pre-cerebellin-like protein [10] and haptoglobin [22]. For some of these, there is evidence that plasma concentrations increase as a consequence of increases in gene expression in the liver [9]. However, there are examples of inducible proteins whose plasma concentrations do increase in fish. For example, CRP increased 3-fold over normal serum levels within 24–48 h following i.p. injection of rainbow trout with *Vibrio anguilarum* [16] and 18-fold after formalin exposure [23], but these changes are minor compared to those expected for CRP induction in some mammals.

There are various opportunities for monitoring health and disease status in fish populations if suitable plasma APP responses can be demonstrated above background variability. With the availability of improved proteomics approaches, many more plasma proteins can be assessed [9,24,25]. In the present report we described several plasma proteins from rainbow trout that meet most criteria of positive APP that increase subsequent to an inflammatory response induced by bacterial products.

2. Materials and methods

2.1. Trial # 1. Individual fish bled multiple times

2.1.1. Animals

Rainbow trout were obtained from Rainbow Springs Hatchery, Thamesford Ont., Canada, and raised at the Central Animal Care Facility of the Ontario Veterinary College, University of Guelph. Fifteen healthy trout (450–550 g) were maintained individually in separate 60 L flow-through water tubs at 9–11 °C and fed a commercial pelleted diet (Martin Mills, Elmira, Ont., Canada) at 2% body weight every second day.

2.1.2. Vaccine and lipopolysaccharide

The Lipogen Triple J vaccine (Aqua Health Ltd, Charlottetown, Prince Edward Island, Canada) contains formalin-inactivated cultures of *A. salmonicida*, *Vibrio anguilarum*, *V. ordali*, and *V. salmonicida* and oil adjuvant. Purified smooth and rough lipopolysaccharide (LPS) [26] was isolated from an auto-agglutinating virulent strain of *A. salmonicida* (B18:94) previously used [24]. The B18:94 strain of