LOCAL INDUCTION OF IGT RESPONSES TO PATHOGENS AND MICROBIOTA IN THE GILL OF RAINBOW TROUT

Zhen Xu[1,2], David Parra[3], Fumio Takizawa[1], Daniela Gómez[1], Louise von Gersdorff Jørgensen[4], Scott LaPatra[5], J. Oriol Sunyer[1,*]

[1]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA
[2]Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei, 430070, China
[3]Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Barcelona, Spain
[4]Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiskberg DK-1870, Denmark
[5]Research Division, Clear Springs Foods Inc., P O Box 712, Buhl, Idaho 83316, USA

Abstract

Gas exchange structures are critical for acquiring oxygen, but they also represent portals for pathogen entry. Local mucosal immunoglobulin (Ig) responses against pathogens in specialized respiratory organs have only been described in tetrapods. We have previously shown that IgT is an Ig specialized in gut and skin mucosal immunity. Thus, we tested the hypothesis that IgT might play a pivotal role in mucosal immunity of teleost gills. In this study, we provide the first structural and functional characterization of all teleost Igs, including secreted IgD, IgM and IgT at a mucosal surface of a teleost fish. Here we show that IgT^+ B cells represent the major B cell subset in the gill filaments. In contrast to reported results by others, we found that all gill B cells expressing surface IgM, also expressed surface IgD and that the percentage of B cells solely expressing either IgD or IgM was negligible. We found that the majority of bacterial microbiota in the gill mucosa is coated with IgT and, to a much lesser degree with IgM and IgD. More crucially, significant specific-IgT immune responses against *Ichthyophthirius multifiliis* (Ich) and *Flavobacterium columnare* were measured in the gill mucus, while pathogen-specific IgM responses were almost exclusively detected in the serum. Pathogen-specific IgD titers were absent both in gill mucus and serum. Importantly, we found significant IgT^+ B-cell proliferative responses in the gill but not in the spleen or head kidney of fish that survived Ich infection. Moreover we also found that Ich- and *F. columnare*-specific IgT titers were locally produced by gill explants of survivor fish while they were absent in the spleen or head kidney explants of the same animals. In addition to showing that IgT is the main Ig player in gill mucosal immunity, the observed generation of local IgT^+ B cell proliferative and pathogen-specific IgT responses in the gills provides the first demonstration of locally induced B cell and secretory Ig responses in the mucosa of a teleost. Moreover, this represents the first study in which a bacterial pathogen is shown to induce dominant IgT responses in a fish mucosal site, thus strongly suggesting that IgT is induced by a variety of pathogens in addition to parasites. Our findings also have special relevance from an applied perspective as they may lead to the development of fish vaccines and immunostimulants that have the capacity to induce gill IgT mucosal immune responses.

KEYWORDS
IgT, *Ichthyophthirius multifiliis*, *Flavobacterium columnare*, microbiota, gill

*Corresponding author. TEL.: 215-573-8592; Fax number: 215-898-7887
E-mail: sunyer@vet.upenn.edu