ELSEVIER

Fish & Shellfish Immunology 23 (2007) 991–1002

Fish & Shellfish Immunology

www.elsevier.com/locate/fsi

Characterization of antisera raised against stickleback (*Gasterosteus aculeatus*) MHC class I and class II molecules

J.P. Scharsack*, M. Kalbe, H. Schaschl [1]

Department of Evolutionary Ecology, Max Planck Institute for Limnology, August-Thienemann-Str. 2, 24306 Ploen, Germany

Received 31 January 2007; revised 6 March 2007; accepted 16 March 2007
Available online 24 March 2007

Abstract

The three-spined stickleback (*Gasterosteus aculeatus*) is an important model organism for investigations on the maintenance of polymorphism of the major histocompatibility complex (MHC) of vertebrates. Analysis of functional aspects of MHC diversity in stickleback would benefit from the availability of MHC specific reagents. Here we characterize antisera raised against recombinant fusion proteins of stickleback MHC class I alpha and class II alpha and beta. Western blot analysis using recombinant proteins confirmed the specificity of the antisera. In brain and muscle preparations, neither of the MHC types was detectable. High levels of each MHC receptor type were observed in gills and spleen and lower levels in head kidneys. In histological sections of gills, epithelial cells of primary and secondary lamellae stained positive with MHC class I antiserum, while single, scattered cells stained positive for MHC class II. In sections of spleen and head kidney, considerable numbers of cells positive for either MHC type were detected. Molecular weight shift in SDS-PAGE after deglycosylation of MHC class I alpha and class II beta confirmed the predicted glyco-protein character of the molecules. The majority of MHC II alpha was not glycosylated; only a small fraction of MHC II alpha was susceptible to deglycosylation. This suggests differential expression of the two stickleback MHC II alpha genes (Gaac-DAA, Gaac-DBA) only one of which (Gaac-DBA) has a site for N-linked glycosylation.

Keywords: *Gasterosteus aculeatus*; MHC class I; MHC class II; ECL; N-glycosylation; Western blot; Immunohistology

1. Introduction

Like all jawed vertebrates, bony fish are endowed with the major histocompatibility complex (MHC) receptor family, subdivided into MHC class I and MHC class II molecules [1,2]. In vertebrates both types of receptors are characterized by a highly polymorphic peptide-binding grove in which self and non-self peptides are presented to T-cells [3,4]. In analogy to mammals, MHC class I receptors in teleosts present peptides from intra-cellular pathogens (e.g. viruses) to cytotoxic $CD8^+$ T-cells, which kill infected cells [5]. Receptors of MHC class II present peptides from extra-cellular pathogens (e.g. bacteria, parasites) to $CD4^+$ T-helper cells that trigger the activation of B-cells resulting

* Corresponding author. Tel.: +49 4522 763 256; fax: +49 4522 763 310.
E-mail addresses: scharsack@mpil-ploen.mpg.de (J.P. Scharsack), kalbe@mpil-ploen.mpg.de (M. Kalbe), h.schaschl@klivv.oeaw.ac.at (H. Schaschl).
[1] Present address: Konrad Lorenz Institute for Ethology, Savoyenstrasse 1a, A-1160 Vienna, Austria.

1050-4648/$ - see front matter
doi:10.1016/j.fsi.2007.03.011

in an antibody mediated specific immune response and memory [6−8]. Based on the presence of functional T-helper cell activities and MHC class II molecules, comparable mechanisms may exist also in teleosts [9].

In three-spined sticklebacks (*Gasterosteus aculeatus*), genes of MHC class I and MHC class II [10,11] are highly polymorphic [12−16]. Therefore, sticklebacks have recently become a prime model organism for the investigation of MHC diversity and its consequences on immunocompetence and sexual selection [17−23]. In addition, the genomic organization of both MHC class I and class II have been studied recently [13,16]. In these studies, a bacterial artificial chromosome (BAC) library, which was created from a single stickleback, was screened for clones containing MHC class I and class II genes. In one study the presence of potentially nine MHC class I genes was revealed in the analysed BAC library [16]. In addition, a single BAC clone was sequenced and showed the presence of three MHC class I genes arranged in tandem repeat in the same transcription orientation. The detected MHC class I genes in the BAC clone were very similar to each other and it has been suggested that these MHC class I genes may have been generated by repeated gene duplication events [16]. The sequence analysis of a BAC clone containing MHC class II genes revealed two sets of paralogous class IIα- and β genes in tandem arrangement, probably generated by recent gene duplication events and inter- and intralocus recombination [13,14]. Furthermore, it has been estimated that sticklebacks contain up to six MHC class IIB genes [10]. The question remains, however, whether all three-spined stickleback individuals possess and express the same number of class I and class II genes.

The number and the degree of polymorphism of MHC class IIβ alleles in stickleback populations, differs among habitats [12,21]. Individual stickleback can have up to 12 alleles of MHC IIβ, but for parasite resistance obviously intermediate numbers (i.e. 5−6) of MHC class IIβ alleles are advantageous [20]. In infection experiments with three parasite species (*Anguillicola crassus*, *Diplostomum pseudospathacaeum*, *Camallanus lacustris*), sticklebacks with intermediate numbers of MHC IIβ alleles were found to have lower parasite loads compared to individuals with more or fewer alleles [20]. In mate choice experiments, female sticklebacks favoured combinations with males, allowing optimal (i.e. intermediate) numbers of MHC IIβ alleles for their offspring [17]. The decision of female stickleback was predictably modified towards the optimal number of MHC class IIβ alleles using MHC class I ligand peptides [24], hinting at the similarities of peptide binding determinants of MHC class I and II molecules. So far, the investigation of MHC diversity in sticklebacks is based on structural analysis of polymorphisms in genomic and expressed DNA sequences. Studies on their functional consequences would benefit from the availability of specific antibodies against stickleback MHC. Here, we characterize antibodies recognizing stickleback MHC class I and MHC class II molecules. Antibodies were generated by immunization of rabbits with recombinant, bacterially expressed fragments of stickleback MHC class Iα, MHC class IIα and IIβ.

2. Materials and methods

2.1. Fish

Three spined sticklebacks (*Gasterosteus aculeatus*) were caught in the lake "Grosser Plöner See" in Northern Germany in winter 2005/2006. They were all offspring from the previous breeding season in spring 2005. Fish were maintained in aquaria with continuous water supply and fed *ad libitum* three times a week with frozen chironomid larvae. To mimic their natural seasonal cycle, fish were kept first in winter conditions (6 °C, 8 h light) then transferred for 2 weeks to spring conditions (12 °C, 12 h light) and subsequently to summer conditions (18 °C, 16 h light). Only fish from summer conditions were used for Western blot and histology analysis. For preparation of cDNA, fish caught in winter 2003/2004 and maintained as above were used.

2.2. Construction of expression vectors

For the production of recombinant fragments of stickleback MHC, sequences were selected that, based on sequence comparisons, are expected to code for conserved, extra-cellular domains. Highly polymorphic areas, likely encoding the peptide binding grooves were excluded (Fig. 1). The MHC fragments were produced in bacteria as N-terminally His-tagged recombinant proteins, using the pQE-30 UA expression vector (Qiagen).

Coding sequences were amplified by polymerase chain reaction (PCR) (Table 1) with cDNA templates generated from a stickleback head kidney. PCR products were ligated into linearized expression vector (pQE-30 UA) with the