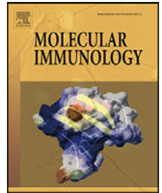
Molecular Immunology 45 (2008) 3107–3116

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm



Identification of MHC class IIβ resistance/susceptibility alleles to *Aeromonas salmonicida* in brook charr (*Salvelinus fontinalis*)

Sébastien Croisetière[a,*], Philippe D. Tarte[a], Louis Bernatchez[b], Pierre Belhumeur[a]

[a] *Département de Microbiologie et Immunologie, Université de Montréal, Montréal, Québec H3C 3J7, Canada*
[b] *Département de Biologie, Université Laval, Québec, Québec G1V 0A6, Canada*

ARTICLE INFO

Article history:
Received 19 February 2008
Received in revised form 7 March 2008
Accepted 10 March 2008
Available online 5 May 2008

Keywords:
Histocompatibility antigens class II
Alleles
Bacterial infections
Disease resistance
Salvelinus fontinalis
Aeromonas salmonicida
Furonculosis
Selection

ABSTRACT

Pathogen-driven selection is believed to be important in the evolution and maintenance of the polymorphism of the major histocompatibility complex (MHC) genes but have been tested for very few vertebrates. In this study, we first investigate by SSCP (single strand conformational polymorphism) the diversity found at the MHC class IIβ gene in a population of brook charr (*Salvelinus fontinalis*) from the Rupert River (Québec, Canada). Secondly, to explore the survival performances conferred by specific alleles and genotypes, individuals from 23 half- and full-sibling families were infected with *Aeromonas salmonicida*, the causative agent of furonculosis. From the initial brook charr population, a total of six MHC class IIβ alleles were identified; four complete and two partial coding sequences that include the complete polymorphic β1 domain. One allele, *Safo-DAB*0101*, was significantly associated with resistance against *A. salmonicida*. In addition to homozygotes for this allele, its resistance effect was also detected in heterozygotes for two specific genotypes. Other allelic combinations, namely heterozygous genotypes *Safo-DAB*0201/*0301* and *Safo-DAB*0301/*0401* were significantly associated with increased susceptibility to furonculosis. Given that its frequency was relatively low (0.0873), the negative frequency-dependent selection hypothesis could explain the advantage associated with the allele *Safo-DAB*0101* over the other alleles and highlight the importance of this mechanism to sustain variation at the MHC in brook charr.
© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

The major histocompatibility complex (MHC) class I and class II genes encode cell-surface proteins specialised in the presentation of self- and non-self-antigen peptides to T-lymphocytes in the adaptive immune system. These represent the most polymorphic genes known to date, with multiple loci and high allelic diversity at each of these loci (Trowsdale and Parham, 2004). Unlike the situation in other vertebrates, the two classical MHC regions, class I and class II, are not found in a complex in bony fishes (Bingulac-Popovic et al., 1997; Sato et al., 2000). For this reason, the expression of “major histocompatibility” (MH) genes is more appropriate in teleosts (Dixon and Stet, 2001). Studies have shown that rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) express a single “minimal, essential” gene of the MH class I (Aoyagi et al., 2002; Grimholt et al., 2002) and class II (Glamann, 1995; Langefors et al., 2000). Also, MH class IIα and class IIβ loci are genetically linked in Atlantic salmon (Stet et al., 2002).

The evolution and maintenance of the polymorphism of the MHC genes can be attributed to two major types of mechanisms: the pathogen-driven and reproductive mechanisms (Aguilar and Garza, 2007; Bernatchez and Landry, 2003). Two main hypotheses have been proposed to explain the pathogen-driven selection: the overdominance and the negative frequency-dependent selection hypothesis. In the overdominance model (or heterozygous advantage), the heterozygous individuals are assumed to present a broader range of pathogen-derived antigens due to a larger number of different MHC molecules and have increased fitness relative to homozygous (Hughes and Nei, 1989). The second model of negative frequency-dependent selection (or rare-allele advantage) argues that individuals bearing low-frequency alleles have an advantage because of the limited co-evolution of the pathogens facing these MHC alleles (Takahata and Nei, 1990; Slade and McCallum, 1992). A third mechanism implicates selection that fluctuates in time and/or space (Hedrick, 2002).

The implications of different MHC class I and class II alleles in disease resistance or susceptibility have been documented previously (e.g. Zekarias et al., 2002; Gebe et al., 2002; Singer et al., 1997; Carrington and O’Brien, 2003; Shiina et al., 2004). In fish, the functional implications of MH polymorphism on the resistance against or susceptibility to infectious diseases have essentially

* Corresponding author. Tel.: +1 514 343 6111x1432; fax: +1 514 343 5701.
E-mail address: sebastien.croisetiere@umontreal.ca (S. Croisetière).

0161-5890/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2008.03.007

all been investigated in Atlantic salmon (*S. salar*). Langefors et al. (2001) were the first to establish a correlation between survival probability and three different MH class IIβ alleles following *Aeromonas salmonicida* exposure. Their observations were confirmed by Lohm et al. (2002) in an experiment where the effect of non-MH background genes could be controlled. Another study identified significant associations between resistances towards infectious diseases caused by both bacterial or viral pathogens and MH class I and class II alleles (Grimholt et al., 2003). It is noteworthy that different alleles were identified as associated with resistance to each pathogen. More recently, a combination of particular MH class I and class II alleles has been associated with resistance or susceptibility to infectious salmon anaemia virus (ISAV) (Kjoglum et al., 2006). Finally, results that could be interpreted as the manifestation of a disease-mediated natural selection have been observed for the MH class II locus (de Eyto et al., 2007).

A. salmonicida, a Gram-negative, facultatively anaerobic, rod-like bacterium, is an invasive pathogen capable of surviving and replicating within intraperitoneal macrophages and non-phagocytic cells (Daly et al., 1996; Garduno et al., 2000). This economically important pathogen is the causative agent of furonculosis that has a strong impact on the survival of salmonids. Previous studies have demonstrated an important genetic component associated with resistance to this pathogen (Perry et al., 2004; Gjedrem, 2000). Brook charr (*Salvelinus fontinalis*) is an important endemic salmonid fish from eastern North America, but there has been no research regarding the MH genes and their performance against *A. salmonicida,* although a heritable genetic basis for resistance to furonculosis has been evidenced in this species (Perry et al., 2004). In this study, our aims were (i) to document the diversity of alleles at the MH class IIβ gene of brook charr and (ii) for the first time, to document survival conferred by specific alleles and/or genotypes following exposure to furonculosis in a fish other than Atlantic salmon.

2. Materials and methods

2.1. Fish and challenge test

Wild brook charr (*S. fontinalis*) originating from the Rupert River and associated waterways were maintained at the LARSA (LAboratoire Régional des Sciences Aquatiques; Université Laval). This strain is of particular interest for aquaculture because it demonstrates rapid growth and large adult size that may exceed 5 kg (Sutton et al., 2002). Fish kept at LARSA were used to generate 23 half- and full-sibling families as previously described (Perry et al., 2004). This allowed us to take into account the effect of genetic background in interpreting the putative role of MH alleles in resistance to furonculosis. To identify individuals of interest for breeding, adult brook charr were first genotyped at the MH class IIβ locus by SSCP (single strand conformational polymorphism). Fish were exposed to a virulent strain of *A. salmonicida* by a bath immersion method (Dautremepuits et al., 2006; Perry et al., 2004; Lutwyche et al., 1995) at 1×10^6 bacteria/ml previously determined to be the LD_{50} at 72 h. Individual survival was recorded every 4 h for the following 90-h period. No mortality was observed in control fish. Dead and surviving individuals were collected and SSCP genotyped at the MH class IIβ locus. The final experimental dataset contained the surviving time of 860 genotyped individuals for statistical analyses.

2.2. MH class IIβ genotyping of brook charr

Genomic DNA from 63 adult brook charrs was extracted from fin adipose for genetic analysis. The different MH class IIβ alleles were first identified using SSCP. A 315–318 bp fragment containing the complete MH class IIβ exon 2, coding for the polymorphic β1 domain of the protein, was amplified by PCR (polymerase chain reaction) using intronic sense primer SP4501 (5′-CCTGTATTTATGTTCTCCTTTC-3′) and antisense primer SP4502 (5′-TAAGTGTTGCTACGGAGCC-3′) in a thermocycler (Biometra). PCR was carried out in a total volume of 50 μl containing 20–40 ng of genomic DNA, 0.2 mM dNTPs, 8 μl of 10× PCR buffer (200 mM Tris–HCl pH 8.4, 500 mM KCl), 2 mM $MgCl_2$, 5 pmol each of SP4501 and SP4502 primers, 1 unit of *Taq* DNA polymerase. The following PCR conditions were used: denaturation for 3 min at 94 °C followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 47 °C and extension for 1 min at 72 °C. The final extension was for 10 min at 72 °C. Three microliters of formamide were added to 9 μl of the PCR products which were then loaded onto a non-denaturing acrylamide gel (10% 49:1 acrylamide:bis-acrylamide, 5% glycerol and 1× TBE) for a 2.5 h and 200 V migration in a Mini-protean III tank (Bio-Rad) in an ice-cold water bath. The procedure resolves alleles into discrete bands that were finally revealed on the gels by silver staining (Budowle et al., 1991).

2.3. RNA isolation from peripheral blood leukocytes (PBL) of brook charr

RNA from PBL of brook charr was extracted to assure the identification of expressed MH class IIβ alleles. Fish were anesthetised with 0.1% of tricaine methanesulfonate (MS-222) (Sigma). The blood was collected from the caudal vein using a heparinised 3 ml syringe with a 21G1 needle (Becton Dickson) and added to 1 volume of ice-cold PBS containing 10 U/ml heparin sodium salt (Sigma). The cellular suspension (1 ml) was then spun at 500 × *g* for 5 min at 4 °C and the buffy layer and plasma transferred to a new Eppendorf. The PBL were then pelleted and suspended in Trizol (Invitrogen) for total RNA extraction according to the manufacturer's protocol.

2.4. Reverse transcriptase (RT)-PCR from PBL

Total RNA (500 ng) from fish of different genotypes was reverse transcribed using SuperScript II reverse transcriptase (Invitrogen), oligo$(dT)_{12-18}$ primer (Invitrogen) and RNAseOUT (Invitrogen) according to the manufacturer's protocol. The complete open reading frame of MH class IIβ was obtained by PCR using primers in the 5′-UTR (P472; 5′-CAGCAGAGGAACATGTCGATG-3′) and the 3′-UTR (P473; 5′-TTTCTGCTGCAGATTCAGCA-3′) designed from sequences at positions conserved between Atlantic salmon and rainbow trout (Syed et al., 2003). The reactions were carried out on homozygous individuals for product homogeneity. PCR was carried out in a total volume of 50 μl containing 0.25 μl of cDNA from a 1/20 dilution of the RT reaction, 0.2 mM dNTPs, 5 μl of 10× Expand High Fidelity buffer (Roche), 2 mM $MgCl_2$, 5 pmol each of P472 and P473 primers, 2.6 units of Expand High Fidelity enzyme mix (Roche). The following PCR conditions were used: denaturation for 3 min at 94 °C followed by 40 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 45 °C and extension for 1 min at 72 °C. The final extension was for 10 min at 72 °C. Sequencing of the PCR products was realized on a 3730 DNA analyser (Applied Biosystems).

2.5. Data analysis

Alignment of the deduced amino acid sequence of MH class IIβ peptide was performed using ClustalX (Thompson et al., 1994). Sequences used for comparison and their GenBank™ accession numbers were as follows: *O. mykiss*: U20943 (Glamann, 1995); *S. salar*: X70166 (Hordvik et al., 1993); *Salmo trutta*: AF296398 (Shum et al., 2001); *Cyprinus carpio*: Z49064 (Ono et al., 1993); *Brachydanio*

Download English Version:

https://daneshyari.com/en/article/2832644

Download Persian Version:

https://daneshyari.com/article/2832644

Daneshyari.com