Experimental evidence for major histocompatibility complex-allele-specific resistance to a bacterial infection

Lohm, Jakob; Grahn, Mats; Langefors, Åsa; Andersen, O; Storset, A; von Schantz, Torbjörn

Published in:
Royal Society of London. Proceedings B. Biological Sciences

DOI:
10.1098/rspb.2002.2114

2002

Link to publication

Citation for published version (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
Experimental evidence for major histocompatibility complex-allele-specific resistance to a bacterial infection

Jakob Lohm1*, Mats Grahn2, Åsa Langefors1, Øivind Andersen3, Arne Storset4 and Torbjörn von Schantz1

1Department of Animal Ecology, Ecology Building, Lund University, SE-223 62 Lund, Sweden
2Södertörn University College and Department of Bioscience, Karolinska Institute, Box 4101, SE 141 04 Huddinge, Sweden
3AKVAFORSK, The Institute of Aquaculture Research, PO Box 5010, N-1432 Ås, Norway
4Aqua Gen AS, N-6600 Sunndalsora, Norway

The extreme polymorphism found at some major histocompatibility complex (MHC) loci is believed to be maintained by balancing selection caused by infectious pathogens. Experimental support for this is inconclusive. We have studied the interaction between certain MHC alleles and the bacterium Aeromonas salmonicida, which causes the severe disease furunculosis, in Atlantic salmon (Salmo salar L.). We designed full-sibling broods consisting of combinations of homozygote and heterozygote genotypes with respect to resistance or susceptibility alleles. The juveniles were experimentally infected with A. salmonicida and their individual survival was monitored. By comparing full siblings carrying different MHC genotypes the effects on survival due to other segregating genes were minimized. We show that a pathogen has the potential to cause very intense selection pressure on particular MHC alleles; the relative fitness difference between individuals carrying different MHC alleles was as high as 0.5. A co-dominant pattern of disease resistance/susceptibility was found, indicative of qualitative difference in the immune response between individuals carrying the high- and low-resistance alleles. Rather unexpectedly, survival was not higher among heterozygous individuals as compared with homozygous ones.

Keywords: major histocompatibility complex class II; disease resistance; alleles; bacterial infection

1. INTRODUCTION

Major histocompatibility complex (MHC) molecules have a central role in the adaptive immune system by presenting foreign peptides to T cells. The evolution and maintenance of the extraordinary polymorphism of the genes encoding MHC molecules are generally attributed to balancing selection caused by infectious pathogens (Clarke & Kirby 1966; Takahata et al. 1992; Apanius et al. 1997). So far, experimental infections in congenic mice and other inbred animal models provide compelling evidence that MHC genes affect host resistance to infectious agents (Apanius et al. 1997). However, background genes (including non-immune system genes) appear to have an important role in MHC-dependent parasite resistance where a particular MHC haplotype may be associated with either resistance or susceptibility to a given pathogen depending on the genetic background of the host (Apanius et al. 1997; Medina & North 1998). Hence, it is still a contentious issue to what extent the results from inbred laboratory strains can be extrapolated to outbred populations (Apanius et al. 1997), where evidence is solely based on correlative data with no control for effects of non-MHC genes (Briles et al. 1983; Hill et al. 1991; Thursz et al. 1997; Paterson et al. 1998; Carrington et al. 1999; Langefors et al. 2001).

Unlike the situation in other vertebrates, in bony fishes the two classical MHC regions, class I and class II, are not linked (Bingulac-Popovic et al. 1997; Flajnik et al. 1999; Sato et al. 2000). Furthermore, studies in the Atlantic salmon (Salmo salar L.) have shown that only a single MHC class II B locus exists in this species (Langefors et al. 2000). These two features of the Atlantic salmon MHC make it an ideal species to test interactions between infectious pathogens and disease resistance caused by specific MHC class II B alleles.

Furunculosis in salmonids is caused by the bacterium Aeromonas salmonicida. It is an infectious disease that has a strong impact on survival in salmonids and in Atlantic salmon. Macrophage-like cells have been shown to regulate the expression of MHC class II mRNA in response to lipopolysaccharide antigen from A. salmonicida (Koppang et al. 1999). Furthermore, selection experiments have revealed a substantial genetic component to resistance against furunculosis in fishes (Gjedrem 2000; Marsden et al. 1996).

In our previous survey (Langefors et al. 2001) conducted on juveniles from an outbred hatchery population of Atlantic salmon (Gjedrem 2000), correlations were found between survival probability and three different MHC class II B alleles (out of nine) following controlled infection with A. salmonicida salmonicida. One of these three alleles was associated with reduced survival probability and the other two with increased survival (Langefors et al. 2001). Three years later, in the autumn of 1998, when
the uninfected siblings of these fishes from our previous survey became sexually mature, their MHC class II B genotype was determined and they were selectively mated in order to create different MHC genic groups combining high- and low-resistance alleles within full-sibling families. The offspring from these matings, carrying controlled MHC genotypes and on average the same genetic background within each group because they consist of full-sibling families, were then infected with furunculosis and their survival was monitored. Through this experiment, we were able to study the effect of specific MHC alleles on disease resistance while excluding potential effects of family. We describe here the outcome of the experiment.

2. MATERIAL AND METHODS

(a) Experimental fishes

Fishes belonging to a large population originating from a number of wild Norwegian populations maintained under farming conditions for six generations (since 1974) where selective breeding has mainly focused on increased growth rate and delayed sexual maturation (Gjedrem 2000) were reared by Aqua Gen AS, Norway. Norway is not considered to be a furunculosis endemic area (Johnsen & Jensen 1994). Pedigree analysis (data not shown) of individuals used to create the full-sibling families in this experiment shows that, in five generations (starting with wild fishes caught in 1974), there have been no matings between known relatives within their parental lineages (except for one case; a sibling mating in the second generation in one of the families), minimizing the likelihood that any non-random associations between the studied MHC gene and other potentially disease controlling loci were generated by inbreeding. To exclude the possibility that our captive population was inbred due to non-random sampling in the founding generation, the genetic variation within the captive population was compared with that of two Baltic salmon populations, the Kalixälven and Dalälven populations. This was done by the use of the amplified fragment length polymorphism (AFLP) technique (Vos et al. 1995), which is a DNA fingerprinting method based on the PCR. The Dalälven population is a non-captive cultured population that has been maintained by culturing since the 1950s to compensate for the loss of natural spawning sites caused by dams built for hydropower production. The Kalixälven population is one of the few remaining unmanaged naturally reproducing Swedish salmon populations.

AFLP analyses were performed as described elsewhere (Vos et al. 1995), with the modification that the EcoRI selective primers were labelled with fluorescein (Life Technoligies), on eight randomly selected individuals from each of the three populations using five sets of selective amplification primers (EcoRI + TAG/MseI + CAC, EcoRI + TAG/MseI + CAG, EcoRI + TCT/MseI + CGT, EcoRI + TCT/MseI + CGG and EcoRI + TGA/MseI + CGC). Fragments were analysed in a Vistra Fluorimager and generated a total of 255 markers. There was no significant difference in the number of polymorphic markers between the three populations ($\chi^2_{2\, df} = 0.36, p = 0.83$). Marker polymorphism was 11.4% in Norway (28 out of 245 markers), 13.2% in Kalixälven (33 out of 250 markers) and 12.5% in Dalälven (31 out of 248 markers) populations.

(b) Artificial mating and furunculosis challenge test

To arrange the preferred matings 321 individually tagged (PIT-tag, Trovan) adults were screened for their MHC class II

Figure 1. MHC class II B allele frequencies in 321 adult Atlantic salmon analysed with DGGE (Langefor et al. 2000) from the parental generation of which 10 were used to create desired family groups. The h bar includes alleles b and c; these alleles were not separated in this particular study (Langefor et al. 2000).
Table 1. Survival distribution and genotypes of Atlantic salmon from five families 18 days after infection with *A. salmonicida*. (The numbers of *e/e* and *j/j* homozygous individuals are given within parentheses. Two matings were *e/j* × *e/j*, *n* = 40 and *n* = 17 offspring, respectively. Two matings were *e/j* × +, *n* = 38 and *n* = 15. One mating was *e/j* × *j/j*, *n* = 40.)

<table>
<thead>
<tr>
<th></th>
<th><em>e/+</em></th>
<th><em>e/j</em></th>
<th><em>j/+</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>before selection</td>
<td>44 (11)</td>
<td>35</td>
<td>71 (28)</td>
</tr>
<tr>
<td>dead</td>
<td>26 (7)</td>
<td>23</td>
<td>55 (22)</td>
</tr>
<tr>
<td>surviving</td>
<td>18 (4)</td>
<td>12</td>
<td>16 (6)</td>
</tr>
<tr>
<td>proportional survival</td>
<td>0.41</td>
<td>0.34</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Figure 2. Kaplan–Meier plots (SPSS 1998) of the survival of different MHC class II B genotypes in Atlantic salmon from five different families after infection (day 0) with *A. salmonicida*. (a) Kaplan–Meier plots of the survival of *e/+* (open circles), *e/j* (filled squares) and *j/+* (filled triangles) fishes. The survival probability for the *j/+* individuals was significantly different from the *e/+* and the *e/j* individuals (see § 3). (b) Kaplan–Meier plots of the survival of *e/e* (open circles), *e/j* (filled squares) and *j/j* (filled triangles) fishes from the two *e/j* × *e/j* matings. The survival probability for the *j/j* individuals was significantly different from the *e/e* and the *e/j* individuals (see § 3). (c) Kaplan–Meier plots of the survival of MHC class II B heterozygous (open circles) and homozygous (filled squares) fishes.

3. RESULTS AND DISCUSSION

The mortality peaked at day 16 and the challenge test was terminated two days later when the overall accumulated mortality was 69.7%. The allele frequencies within the five families did not differ from Mendelian expectations (χ² = 2.78, *p* = 0.25, *n* = 300 alleles; table 1) indicating that there was no selection against any particular allele (*e, j* or +) from fertilization until the experiment started 10 months later. During the experiment (figure 2a–c), 104 individuals from our experimental broods died and the remaining 46 were scored as survivors (table 1). Ten per cent of all infected fishes dying during the furunculosis outbreak between day 10 and 18 were examined bacteriologically and *A. salmonicida salmonicida* was re-isolated in all cases. We predicted, based on our previous results (Langefors et al. 2001), that individuals carrying allele *e* would have a higher survival probability than individuals carrying allele *j*. The risk of dying of infection was significantly higher for *j/+* compared with *e/+* individuals (Relative Hazard (RH) = 1.95, *p* = 0.008, *n* = 115; figure 2a) and with *e/j* individuals (RH = 1.81, *p* = 0.043, *n* = 106; figure 2a). However, the survival rate for the heterozygotes (*e/j*) was not significantly different from that for the *e/+* genotype (RH = 1.57, *p* = 0.257, *n* = 79; figure 2a). Separate analyses of the two families that only carried the two specific alleles (sibling genotypes: *e/e, e/j* and *j/j*) showed the same result, with even more pronounced differences. The survival probability of the *j/j* fishes was significantly different from that of the *e/e* (RH = 3.35, *p* = 0.008, *n* = 30; figure 2b) and *e/j* (RH = 2.11, *p* = 0.029, *n* = 46; figure 2b) genotypes but there was no difference in survival between *e/e* and *e/j* individuals (RH = 1.49, *p* = 0.379, *n* = 38; figure 2b). These phenotypic differences are congruent with a dominant effect on disease resistance linked to allele *e* that is stronger than the dominant susceptibility linked to allele *j*. The *j/+* fishes did not survive better than the *j/j* fishes (RH = 0.41, *p* = 0.123, *n* = 71; figure 2a,b), indicating that the increased susceptibility of the *j* allele is not due to a deficiency of antigen presentation in homozygous *j/j* individuals that could have been compensated for by the presence of a neutral allele in the heterozygotes. Instead, this co-dominant pattern of disease resistance/susceptibility is indicative of a qualitative difference in the immune response between individuals carrying the *j* and the *e* alleles, perhaps mediated by a difference in how the alleles regulate the T-cell response. Because there was no significant difference in survival between homozygotes and heterozygotes (RH = 0.95, *p* = 0.822, *n* = 150; figure 2c) and the difference in survival between genotypes was due to the *j/+* group compared with the *e/j* and *e/+* groups, the results demonstrate that resistance or susceptibility to furunculosis infection in Atlantic salmon is associated with specific MHC class II B alleles rather than MHC heterozygosity.

Interactions between MHC and other genes is generally believed to decrease the relative importance of the MHC for disease resistance and the effect of genetic background is often suggested as an explanation to the difficulties in demonstrating MHC effects in outbred populations (Apanius et al. 1997). While this experiment was done in an outbred genetic background, where the genetic background within each group is on average constant because
the groups consist of full-sibling families, there was a strong effect of the MHC. The difference between the MHC class II B e and j alleles can be expressed in terms of relative fitness (w) calculated from the proportion surviving j/+ individuals divided by the proportion surviving e/+ individuals (table 1), giving 0.23/0.41 = 0.55. The corresponding selection coefficient (s = 1 − w) against the j allele would then become 0.45. This calculation however only uses fishes surviving to the end of the experiment and a more accurate estimate of s can be computed from the relative hazard value from the Cox regression analysis, s = 1 − (1/1.95) = 0.49. This value of s is very high compared with previous estimates of the selection coefficient at MHC genes (Edwards & Hedrick 1998). This can be explained by the fact that we do not account for differences over the whole lifespan. Yet, our experiment demonstrates that a pathogen has the potential to cause intense selection pressure on particular MHC alleles. A strong directional selection, if it persists over several generations, is incompatible with the high levels of MHC polymorphism observed in this and other species (Clarke & Kirby 1966; Takahata et al. 1992; Apanius et al. 1997; Langefors et al. 2001; figure 1). Thus, it is probable that the fitness of different MHC alleles differs for different pathogens (Penn & Potts 1999) and shifts over time.

This study clearly shows a strong survival advantage for individuals carrying a high-resistance allele when exposed to a bacterial infection. The design of our experiment permitted us to compare the different MHC class II alleles while holding the effects on survival due to other segregating genes constant by comparing full-sibling families. It also allowed for genetic variation at other unlinked loci making it a more realistic model of a wild population than inbred congenic lines most often used to establish selection pressure on particular MHC alleles. A strong polymorphism (RFLP), denaturing gradient gel electrophoresis (DGGE) and sequencing. Mol. Ecol. 9, 215–219.


Penn, D. J. & Potts, W. K. 1999 The evolution of mating pref-
MHC-allele-specific resistance in salmon  J. Lohm and others  2033

SPSS 1998 Spss, v. 8.0.0. Chicago, IL: SPSS Inc.