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B cell signatures of BCWD-resistant and susceptible lines of rainbow trout: A shift towards more EBF-expressing progenitors and fewer mature B cells in resistant animals

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ABSTRACT

Bacterial cold water disease (BCWD) is a chronic disease of rainbow trout, and is caused by the Gram-negative bacterium *Flavobacterium psychrophilum* (Fp), a common aquaculture pathogen. The National Center for Cool and Cold Water Aquaculture has bred two genetic lines of rainbow trout: a line of *Fp*-resistant trout (ARS-Fp-R or R-line trout) and a line of susceptible trout (ARS-Fp-S, or S-line). Little is known about how phenotypic selection alters immune response parameters or how such changes relate to genetic disease resistance. Herein, we quantify interindividual variation in the distribution and abundance of B cell populations (B cell signatures) and examine differences between genetic lines of naive animals. There are limited trout-specific cell surface markers currently available to resolve B cell subpopulations and thus we developed an alternative approach based on detection of differentially expressed transcription factors and intracellular cytokines. B cell signatures were compared between R-line and S-line trout by flow cytometry using antibodies against transcription factors early B cell factor-1 (EBF1) and paired domain box protein Pax5, the pro-inflammatory cytokine IL-1β, and the immunoglobulin heavy chain mu. R-line trout had higher percentages of EBF^+ B myeloid/ progenitor and pre-B cells in PBL, anterior and posterior kidney tissues compared to S-line trout. The opposite pattern was detected in more mature B cell populations: R-line trout had lower percentages of both IgM^+ mature B cells and IgM-secreting cells in anterior kidney and PBL compared to S-line trout. *In vitro* LPS-activation studies of PBL and spleen cell cultures revealed no significant induction differences between R-line and S-line trout. Together, our findings suggest that selective resistance to BCWD may be associated with shifts in naive animal developmental lineage commitment that result in decreased B lymphopoiesis and increased myelopoiesis in BCWD resistant trout relative to susceptible trout.

1. Introduction

Breeding for improved disease resistance in farm animals has become a major focus in applied immunogenetics. Most disease resistance breeding programs target one or several pathogens having high economic impact (Bishop et al., 2011). Little is known about how breeding for a specific disease resistance phenotype results in correlated changes in immune response parameters and how such changes relate to improved survival following pathogen challenge. Rainbow trout (*Oncorhynchus mykiss*) are a unique resource to address these questions as this species has been domesticated for many years, large numbers of trout are available per cross (1000s), pedigreed lines are available and rainbow trout are a widely used in biomedical research (Thorgaard et al., 2002). Bacterial cold water disease (BCWD) is a common, chronic disease in rainbow trout, and is caused by the Gram-negative bacterium *Flavobacterium psychrophilum* (Fp). Since 2005, the National Center for Cool and Cold Water Aquaculture (NCCCWA) has implemented a rainbow trout selective breeding program to increase survival following BCWD challenge (Hadidi et al., 2008; Leeds et al., 2010; Silverstein et al., 2009). A genetic line that is highly resistant to Fp challenge has been established through family-based selective breeding, designated ARS-Fp-R (or R-line), and in addition, a reference susceptible line, ARS-Fp-S (S-line) is available for phenotypic comparisons. The S-line has a similar founder genetic contribution as the R-line (Hadidi et al.,

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2008), was evaluated at the same age and size, and was selected for one generation for increased susceptibility (Wiens et al., 2013a). When challenged with *Fp* by intraperitoneal injection, the R-line exhibits splenic lower loads (Hadidi et al., 2008) and higher survival by 5–10 days post-infection (Wiens et al., 2013a), suggesting enhanced capacity for pathogen control relative to the S-line. Whether these differences are the result of base-line changes in immunological parameters (i.e. cell numbers) or are manifested following pathogen exposure, or a combination of both possibilities, is unknown.

BCWD typically affects fry and juvenile stages (Barnes and Brown, 2011; Nematollahi et al., 2003), and as such, the disease can manifest itself before the acquired immune system has been fully developed. Many genes and immune proteins are regulated following infection that include serum amyloid A, antimicrobial peptides, MHC I and II, complement components and a number of chemokines and cytokines (Evenhuis and Cleveland, 2012; Henriksen et al., 2014; Langevin et al., 2012; Orieux et al., 2013; Villarroel et al., 2008). Resistance to Fp has been correlated with naive animal spleen size, with Fp-resistant trout having larger spleens, although the functional significance remains unclear (Hadidi et al., 2008; Wiens et al., 2013b). Adaptive immunity (IgM antibody) is generated in convalescent fish, and serum adoptive transfer provides partial protection against experimental challenge (LaFrentz et al., 2002). Based on these observations, mechanisms that provide resistance against Fp infection and/or disease progression could include innate and acquired immune systems and potentially manifested either prior to, or induced following, pathogen exposure.

The developmental pathways of the teleost immune system are not well defined compared to highly studied mammalian systems. However, some important progress has been made recently, as the main developmental and terminally differentiating B cell populations have now been defined in rainbow trout (reviewed in Zwollo, 2011). Trout B lymphoid cell stages are similar to those of the mammalian system, including the earliest stages, common lymphoid Progenitor (CLP)/pro-B and pre-B cell stages, followed by immature B, mature B, plasmablast, and finally, the plasma cell stage. Using flow cytometric analysis, tissue-specific B cell patterns or "B cell signatures" have been defined for the major immune organs in trout, based on combinatorial expression of transcription factors, which provide accurate developmental markers that are highly conserved between species (Barr et al., 2011; MacMurray et al., 2013; Zwollo, 2011). Early developing B lymphoid cells can be characterized by the expression of the transcription factor early B cell factor (EBF) and the recombinase RAG1 (Zwollo, 2011; Zwollo et al., 2008). EBF is first expressed in CLPs, and its expression is highest in large pre-B cells (Lukin et al., 2008, MacMurray et al., 2013; Fig. 1). Late developing B cells (including small pre-B and (im)mature B cells) have low levels of EBF and lack RAG1, but strongly express the transcription factor Pax5.PD (the Pax5 isoform containing a paired domain) and the immunoglobulin heavy chain mu (HCmu), while terminally differentiated plasma cells lack Pax5 and membrane IgM, but secrete high levels of IgM ($HCmu^{++}$; Zwollo et al., 2010, Barr et al., 2011, MacMurray et al., 2013; Fig. 1). Plasmablasts continue to co-express Pax5 and HCmu, and can be detected using proliferation marker EdU (Barr et al., 2011).

In teleosts, including the rainbow trout, the main site for hematopoiesis is the anterior kidney or K1 (Zwollo et al., 2005), and as the main primary immune site, it has the highest concentration of developing B cells (Zapata and Cooper, 1990; Zwollo et al., 2010). It is also believed to be the site for storage of long-lived plasma cells (LLPCs; Bromage et al., 2004, Ma et al., 2013). In contrast, posterior kidney (named K5) possesses both renal and immune tissue, the latter containing mature and activated B cells (Zapata and Cooper, 1990; Zwollo et al., 2010). The trout spleen retains some hematopoietic function in adult animals, as it expresses low levels of RAG1 (Hansen, 1997; Zwollo et al., 2010) and EBF (MacMurray et al., 2013); however, it primarily functions as a secondary immune organ where mature B cells are activated by antigen. As in mammalian species, trout spleen has abundant levels of mature B cells (Bromage et al., 2004; Kaattari and Irwin, 1985; Zapata and Cooper, 1990; Zwollo et al., 2005, 2008). The blood of the rainbow trout is a poorly understood and complex immune site. It contains many (im) mature, IgM^{+} B cells as well as progenitor cells, but very few plasmablasts/

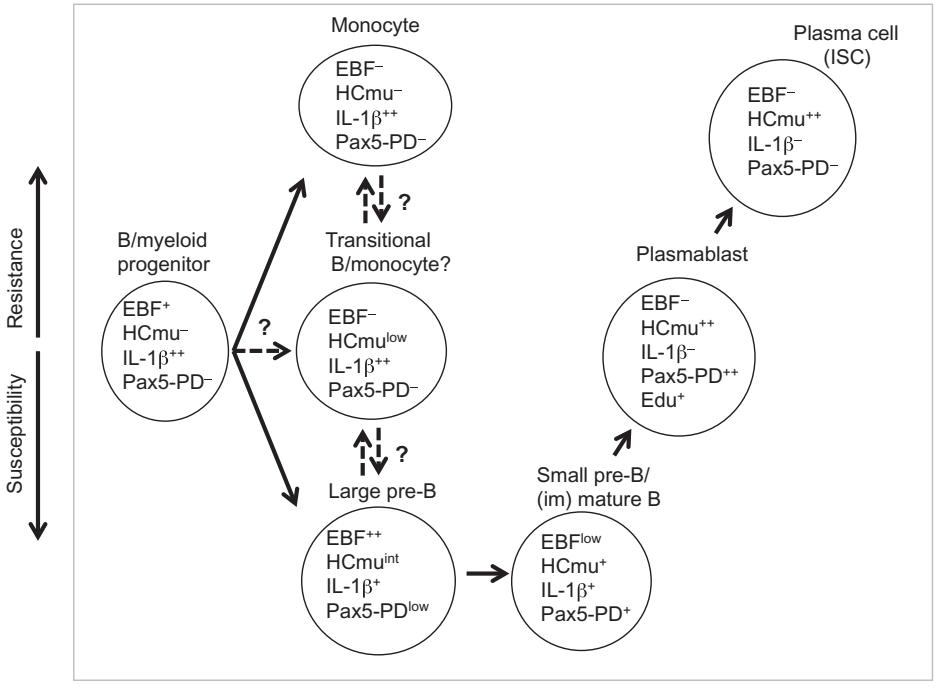

Fig. 1. Predicted phenotype of cells based on immune markers used. EBF, early B cell factor; HCmu, immunoglobulin heavy chain mu, Pax5.PD, Pax5-paired domain-specific. Dotted arrows with question marks indicate hypothetical pathways. Only B cell maturation pathways for the isotype IgM are depicted here.