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Review

Antimicrobial mechanisms of fish leukocytes

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ABSTRACT

Early activation and coordination of innate defenses are critical for effective responses against infiltrating pathogens. Rapid engagement of immune cells provides a critical first line of defense soon after pathogen infiltration. Activation leads to a well-orchestrated set of events that sees the induction and regulation of intracellular and extracellular antimicrobial defenses. An array of regulatory mediators, highly toxic soluble molecules, degradative enzymes and antimicrobial peptides provides maximal protection against a wide range of pathogens while limiting endogenous damage to host tissues. In this review we highlight recent advances in our understanding of innate cellular antimicrobial responses of teleost fish and discuss their implications to cell survival, immunomodulation and death. The evolutionary conservation of these responses is a testament to their effectiveness against pathogen infiltration and their commitment to effective maintenance of host homeostasis. Importantly, recent developments in teleost fish systems have identified novel host defense strategies that may be unique to this lower vertebrate group or may point to previously unknown innate mechanisms that also play a significant role in higher vertebrate host immunity.

Contents

Abbreviations: NADPH, nicotinamide adenine dinucleotide phosphate; PKC, protein kinase C; ROI, reactive oxygen intermediates; PAMPs, pathogen associated molecular patterns; TNF, tumor necrosis factor; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; CpG ODN, CpG oligodeoxynucleotides; Poly I: C, polyinosinic: polycytidylic; MDP, muramyl dipeptide; PKM, primary kidney macrophage; NO, nitric oxide; iNOS, inducible nitric oxide synthase; NF, κBnuclear factor kappa B; TGF, transforming growth factor; cAMP, cyclic 3′,5′-adenosinemonophosphate; NETs, neutrophil extracellular traps; AMPs, antimicrobial peptides.

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1. Introduction

The innate immune system provides a critical first line of defense against invading pathogens. Multi-parametric recognition of pathogen associated molecular patterns (PAMPs) based on well-established receptor families (e.g. TLRs, NLRs) effectively defines microbial intruders and ultimately leads to activation of cell armamentarium designed to kill infiltrating pathogens. Antimicrobial responses are tailored to the type of pathogen, as well as to the location of the pathogen (internalized or external to the cell). Because of their potency and efficiency, these primordial immune defenses have been largely conserved through evolution. For lower vertebrate species like the teleost fish, these innate antimicrobial responses are particularly critical for host survival in light of the reduced repertoire of classical adaptive responses compared to those of mammalian species.

Antimicrobial defenses can be divided into two main categories: intracellular and extracellular. Intracellular defense mechanisms are designed to provide protection against pathogens found within membrane-enclosed structures. These defenses are not limited to killing pathogens that have been internalized (e.g. though phagocytosis), but also provide protection against pathogens that actively enter immune cells as a mechanism of protection from humoral defense mechanisms. In this review, we highlight the role of mechanisms based on superoxide and nitric oxide production as well as phagolysosome fusion for the effective establishment of a toxic degradative environment within teleost fish leukocytes. These soluble products are efficient antimicrobial agents, but their mode of action is generally non-specific (Morel et al., 1991; Nathan and Hibbs, 1991; Stuart and Ezekowitz, 2005). As such, they can be highly toxic to both microorganisms and host cells. Targeted production and release of these antimicrobial molecules into membrane-enclosed structures ensures reduced damage to host phagocytes, while also sequestering the pathogen within a specialized degradative environment.

Extracellular defense mechanisms provide complementary strategies to those described above. Extracellular defenses are targeted towards pathogens within the extracellular space, providing a means for the innate immune system to effectively clear pathogens that have escaped internalization or those that are too large to be internalized. These responses are generally activated by the presence of microbial products or inflammatory mediators and result in the release of antimicrobial factors into the extracellular space. While reactive oxygen and nitrogen intermediates can also be produced extracellularly and thus be considered extracellular defense mechanisms, this review will focus on the antimicrobial contributions provided by degranulation of neutrophilic granules, formation of neutrophil extracellular traps and antimicrobial peptides as representative strategies for the effective defenses against extracellular pathogens. Unlike intracellular defenses, products in neutrophilic granules and antimicrobial peptides are more specifically targeted towards microorganisms and cause little damage to healthy host cells (Zasloff, 1992; Faurschou and Borregaard, 2003).

The mechanisms and effectiveness of teleost cellular responses against microbial challenge are well documented for classical professional phagocytes such as monocytes, macrophages and neutrophils (Neumann et al., 2001; Mathias et al., 2009). These provide clear examples of the potent intracellular (do Vale et al., 2002; Cuesta et al., 2007; Rieger et al., 2010) and extracellular (Palic et al., 2007b; Cuesta et al., 2008a; Forlenza et al., 2008; Mulero et al., 2008) antimicrobial strategies available to the teleost host. There is also increasing evidence that non-classical cells such as B-lymphocytes may fill important roles in early teleost antimicrobial defenses. B cells have recently been described to be phagocytic and to effectively mediate killing of phagocytosed bacteria (Li et al., 2006; Overland et al., 2010; Zhang et al., 2010). Further, phagocytic B cells appear to represent a significant proportion of the phagocytic blood leukocytes in trout, suggesting significant contributions for phagocytic B cells in teleost host defense (Li et al., 2006). It remains unclear whether the presence of phagocytic B cells reflects a requirement for specialization from a lower vertebrate group that relies heavily on innate defense mechanisms for pathogen clearance. Alternatively, this may point to novel innate mechanisms that may have remained conserved within specialized niches in higher vertebrates and are yet to be adequately characterized.

In this review we highlight recent advances in our understanding of antimicrobial responses of teleost fish neutrophils, monocytes, macrophages and B cells and discuss the implications of these antimicrobial responses on survival, immunomodulation and death of these leukocytes. Ultimately, these are critical to the effectiveness of early host antimicrobial responses, the coordination of subsequent adaptive mechanisms, the conservation of host integrity, and the maintenance of homeostasis. For complementary reviews on antimicrobial mechanisms of teleost fish, readers are directed to the following excellent reviews (Secombes and Fletcher, 1992; Neumann et al., 2001; Traver et al., 2003; Plouffe et al., 2005; Magnadottir, 2006; Robertsen, 2006; Alvarez-Pellitero, 2008). Further, this special issue contains additional complementary reviews that highlight exciting recent advances in our understanding of the immune defense mechanisms of teleost fish.

2. Antimicrobial killing mechanisms

2.1. Intracellular mechanisms

2.1.1. Respiratory burst

The respiratory burst was first described in mammalian leukocytes in the 1930s when it was noted that phagocytosis was associated with increased oxygen consumption (Baldridge and Gerard, 1933). It was subsequently found that this increased oxygen consumption- or respiratory burst-resulted in the formation of superoxide anion (Babior et al., 1973) and that this process was catalyzed by NADPH-oxidase, a multi-component enzyme that assembled on the inner surface of the plasma membrane following appropriate activation (Briggs et al., 1975). In mammalian phagocytes, the NADPH-oxidase consists of the catalytic membrane-associated flavocytochrome b_{588}, which is a heterodimer gp91^{phox} (also known as Nox2; phox for *ph*agocyte *ox*idase) and p22^{phox} (Babior, 1999; Robinson, 2009). The remaining three components of NADPH-oxidase – p40^{phox}, p47^{phox}, and p67^{phox} remain complexed in the cytosol until appropriate stimulation is received (Babior, 1999), thereby providing an important control strategy for NADPH-oxidase activation in resting cells.

Phagocyte NADPH-oxidase has only begun to be characterized in teleost fish. Cloning, sequencing and phylogenetic analysis NADPH-oxidase has been described in several teleost fish species, including rainbow trout (*Oncorhynchus mykiss*) (Boltana et al., 2009), Japanese pufferfish (*Takifugu rubripes*) (Inoue et al., 2004), carp (*Cyprinus carpio*) (Mayumi et al., 2008), Atlantic salmon (*Salmo salar*) (Olavarria et al., 2010), zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), and pufferfish (*Tetraodon nigroviridis*) (Kawahara and Lambeth, 2007; Kawahara et al., 2007). Phylogenetic analysis indicates that the radiation of NADPH-oxidase components occurred in a common teleost/mammalian ancestor (Mayumi et al., 2008) and have evolved separately, leading to a clustering of all fish components separate from mammals (Olavarria et al., 2010).

Although the evolutionary divergence observed for NADPH-oxidase has led to a relatively low sequence homology between fish and mammals, the functional domains remain highly homologous (Inoue et al., 2004; Mayumi et al., 2008; Boltana et al., 2009; Olavarria et al., 2010). Fish phox subunits contain all essential inter-