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Review

Heterogeneity of macrophage activation in fish

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ABSTRACT

In this review, we focus on four different activation states of fish macrophages. *In vitro*, stimulation with microbial ligands induces the development of innate activated macrophages whereas classically activated macrophages can be induced by stimulation with LPS in combination with (recombinant) IFNγ. Both types of macrophages show elevated phagocytic activity, expression of pro-inflammatory cytokine genes and radical production. Alternatively activated macrophages require the cytokines IL-4/IL-13 for induction of, among others, arginase activity. Until *in vitro* studies identify the effects of putative IL-4 and IL-13 homologues on fish macrophages, arginase enzyme activity remains the most reliable marker for the presence of alternatively activated macrophages in fish. The best evidence for the existence of regulatory macrophages, associated with the presence of IL-10, comes from *in vivo* studies, for example during parasitic infections of carp. Altogether, differentially activated macrophages in fish largely resemble the phenotypes of mammalian macrophages. However, the presence of fish-specific ligand recognition by TLRs and of duplicated genes coding for proteins with particular activities, poses additional challenges for the characterization of phenotype-specific gene signatures and cell surface markers.

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1. Introduction

Macrophages arise from hematopoietic progenitors which differentiate directly, or *via* circulating monocytes, into subpopulations of tissue macrophages (Geissmann et al., 2010). Resident tissue macrophages of mammalian vertebrates can have various morphologic and phenotypic differences depending on the organ, and include Kupffer cells in the liver, alveolar macrophages in the lung, microglia cells in the central nervous system, osteoclasts in bone tissue and specialized macrophages in the spleen (Gordon and Taylor, 2005). All these types of macrophages are important for the maintenance of homeostasis, including the immune response to pathogens. The recent advancements in our understanding of macrophage development in teleosts, including the growth factors important for the regulation of macrophage development, have recently been summarized (Hanington et al., 2009) and are not part of this review. Rather, we will focus on the so-called macrophage activation states, reflecting the different phenotypes these cells acquire in response to distinct environmental signals. Based on the activation triggers and their resulting effector

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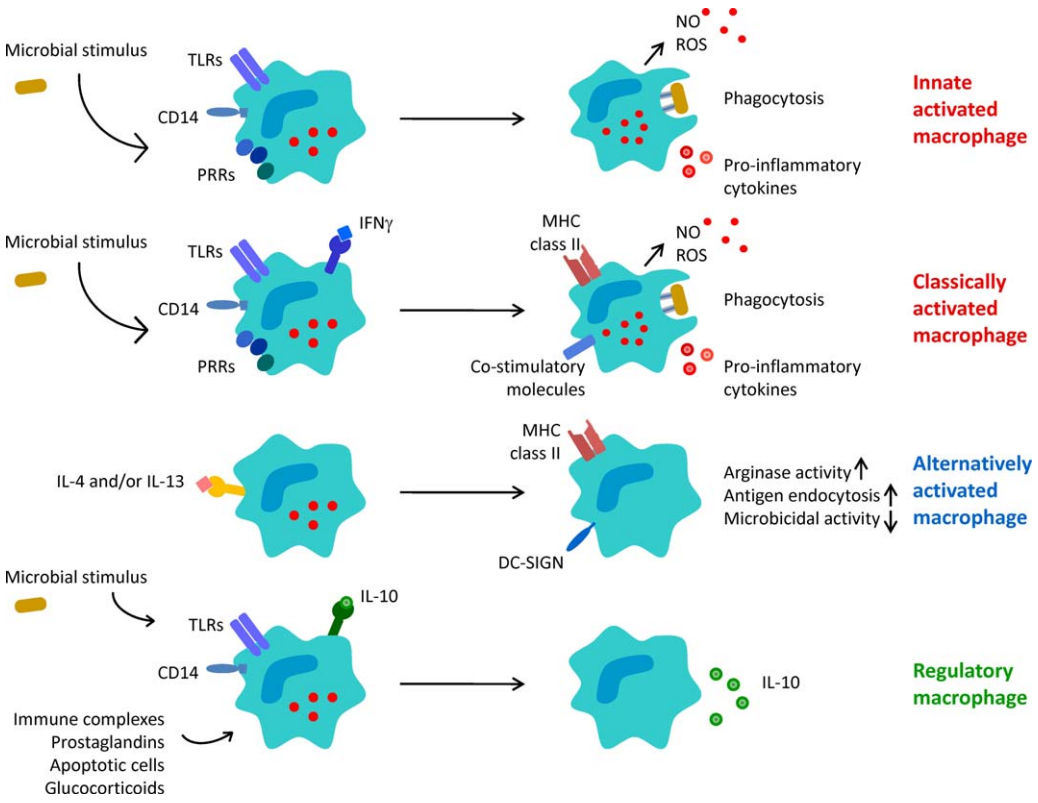

Fig. 1. Activation of macrophages: Microbial stimuli are recognized by macrophages through Toll-like receptors (TLRs), CD14, or other pattern recognition receptors (PRRs). Stimulation with microbial antigens leads to the development of innate activated macrophages with increased phagocytic activity, production of pro-inflammatory cytokines, reactive oxygen species (ROS) and nitric oxide (NO). A microbial stimulus combined with IFNγ induces classically activated macrophages that are characterized by a higher expression of MHC class II and co-stimulatory molecules in addition to the effector functions already described for innate activated macrophages. Alternatively activated macrophages develop in the presence of the cytokines IL-4 and/or IL-13 and express DC-SIGN as well as higher levels of MHC class II molecules. Alternatively activated macrophages have increased arginase activity, antigen endocytosis and decreased microbicidal activity. Regulatory macrophages develop in response to IL-10 or upon stimulation with a microbial stimulus in combination with a second signal that can be, for example, immune complexes. Regulatory macrophages are characterized by the production of high levels of IL-10.

functions and cytokine profile, macrophages have been broadly divided into two types: classically activated macrophages induced in a T helper 1 (T_H1) cytokine environment, and alternatively activated macrophages, induced in a T_H2 cytokine environment (Stein et al., 1992; Goerdt and Orfanos, 1999; Mantovani et al., 2002; Gordon, 2003). Mirroring the T_H1–T_H2 dichotomy, classically activated macrophages have also been termed M1, whereas alternatively activated macrophages have been termed M2 (Mills et al., 2000). More recently, classifications containing more subtypes of macrophage activation states have been introduced to take into account the diversity of macrophage phenotypes that are induced when these cells are exposed to different environmental signals (Mantovani et al., 2004; Mosser and Edwards, 2008).

In this review, we have adopted a definition of four different phenotypes of macrophages (Fig. 1). Innate activation (i) is defined to occur when a macrophage responds to a microbial stimulus alone, whereas classical activation (ii) is defined to require a microbial stimulus plus the presence of the cytokine IFNγ (Dalton et al., 1993). Compared to innate activated macrophages, classically activated macrophages present higher respiratory burst activity and iNOS expression as well as increased antigen presentation and co-stimulation (MHC class II and CD86, respectively) (Gordon and Taylor, 2005). It is worth noting that these activated macrophages, having such potent effector functions, must be kept under tight regulation to prevent them from causing damage to host tissues. We restrict the term alternatively activated macrophages (iii) to macrophages generated in the presence of the T_H2 cytokines IL-4 and/or IL-13. These cells, which have also been termed M2a (Mantovani et al., 2004) or wound healing macrophages (Mosser and Edwards, 2008) are characterized by increased arginase activity, production of proteins for extracellular matrix and polyamines and indirectly counterbalance the activity of innate/classically activated macrophages by metabolizing L-arginine (Gordon and Martinez, 2010), the substrate for iNOS. Macrophages stimulated by Toll-like receptor (TLR) ligands in combination with a second signal that can be, for example, immune complexes, have been termed M2b or type-II, whereas macrophages which develop in response to IL-10, have been termed M2c or deactivating macrophages (Mantovani et al., 2004). Both, M2b and M2c macrophages produce high levels of IL-10 thereby directly contributing to the down-regulation of T_H1 immune responses. In this review, we will use the term regulatory macrophages (iv) for macrophages associated with the presence of IL-10.

It is important to point out that there is not a rigid barrier between these macrophage phenotypes and that, indeed, cells exhibiting characteristic markers from more than one of these "activation states" can be observed (Bronte et al., 2003; Ghassabeh et al., 2006; Mosser and Edwards, 2008). This plasticity of macrophages has added to the confusion regarding the existence of individual macrophage sub-types. Another confounding factor is the dissimilarity between different species; given the fact that macrophages of mouse and man show important differences (Mestas and Hughes, 2004; Gordon and Martinez, 2010) it should not be surprising to observe differences between fish and mouse macrophages, or even between macrophages of different fish species. In this review we will discuss the state of the art on innate and classically activated macrophages as opposed to alternatively activated and regulatory macrophages in teleost fish.