Developmental and Comparative Immunology 58 (2016) 60–67

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

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

Amphibian macrophage development and antiviral defenses

Leon Grayfer [a], Jacques Robert [b, *]

[a] Department of Biological Sciences, George Washington University, Washington, DC, USA
[b] Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY, USA

ARTICLE INFO

Article history:
Received 18 September 2015
Received in revised form
12 December 2015
Accepted 13 December 2015
Available online 15 December 2015

Keywords:
Amphibian
Macrophages
Myelopoiesis
Monopoiesis
Ranavirus
Colony-stimulating factor-1
Interleukin-34

ABSTRACT

Macrophage lineage cells represent the cornerstone of vertebrate physiology and immune defenses. In turn, comparative studies using non-mammalian animal models have revealed that evolutionarily distinct species have adopted diverse molecular and physiological strategies for controlling macrophage development and functions. Notably, amphibian species present a rich array of physiological and environmental adaptations, not to mention the peculiarity of metamorphosis from larval to adult stages of development, involving drastic transformation and differentiation of multiple new tissues. Thus it is not surprising that different amphibian species and their respective tadpole and adult stages have adopted unique hematopoietic strategies. Accordingly and in order to establish a more comprehensive view of these processes, here we review the hematopoietic and monopoietic strategies observed across amphibians, describe the present understanding of the molecular mechanisms driving amphibian, an in particular *Xenopus laevis* macrophage development and functional polarization, and discuss the roles of macrophage-lineage cells during ranavirus infections.

1. Introduction

It is becoming evident that akin to other vertebrate species, amphibians rely heavily on macrophage-lineage cells not only for immune defense, but also for homeostasis and tissue remodeling/resorption (Haislip et al., 2011; Nishikawa et al., 1998). Whereas committed macrophage precursors of other vertebrates reside within designated hematopoietic sites (Bartelmez et al., 1989; Garceau et al., 2010; Kriegler et al., 1994), amphibian species appear to vary in their respective hematopoietic strategies and do not always harbor macrophage precursors within designated sites used by other blood cells for development. This is particularly interesting when considering that many amphibians possess two distinct developmental stages: a typically aquatic tadpole stage and a more terrestrial adult one, each with distinct physiological and immunological requirements. Although metamorphosis is generally rudimentary, or even cryptic in urodelian species (e.g. salamanders, newts), in anuran species it is a major developmental transition between two distinct immune systems [reviewed in (Flajnik et al., 1987; Robert and Ohta, 2009)]. In addition, the different ecological niches occupied by tadpole and adult stages are presumably populated by different pathogens, thus representing unique immunological pressures. As such, metamorphosis is likely to have a profound influence on macrophage development and biology. Macrophage-lineage cells are of particular relevance when considering the alarming increase in the morbidity and mortality of amphibian populations worldwide caused by ranavirus infections [large DNA viruses of the family *Iridoviridae* (Chinchar, 2002; Chinchar et al., 2009; Williams et al., 2005)]. Indeed, there is increasing evidence implicating amphibian macrophages in persistence, evasion and dissemination of ranaviruses, and possibly differences in the interaction of these pathogens with tadpole and adult macrophages [discussed below and reviewed in (Chen et al., 2011; Grayfer et al., 2012)]. Thus, it is imperative that we garner greater insights into the ontogeny and functionality of these amphibian innate immune effectors.

The embryonic origins of amphibian hematopoietic precursors have been described in detail elsewhere (Ciau-Uitz et al., 2014, 2010a) and will be addressed in passing here. This review addresses the current understanding of amphibian hematopoiesis

Abbreviations: Arg-1, arginase-1; CSF-1, colony stimulating factor-1; CSF-1R, colony stimulating factor-1 receptor; CFU, colony forming units; FV3, Frog Virus 3; HSCs, hematopoietic stem cells; IL-34, interleukin-34; M-CSF, macrophage-colony-stimulating factor; NF, Nieuwkoop and Faber; PWM-SCM, pokeweed mitogen-stimulated spleen cell medium; r*Xl*, recombinant *Xenopus laevis*.

* Corresponding author.
E-mail address: Jacques_Robert@urmc.rochester.edu (J. Robert).

http://dx.doi.org/10.1016/j.dci.2015.12.008
0145-305X/

with a focus on myelopoiesis, and it highlights recent notable findings pertaining to the roles of amphibian macrophages during ranavirus infections.

2. Diversified sites of hematopoiesis in amphibians

Vertebrate blood cell precursors differentiate within designated sites of hematopoiesis. Typically, avian and mammalian committed myeloid-lineage progenitors arise from the bone marrow pluripotent populations (Bartelmez et al., 1989; Garceau et al., 2010; Kriegler et al., 1994), whereas teleost fish utilize the head kidney as their designate site of hematopoiesis (Belosevic et al., 2006; Neumann et al., 2000). In amphibians, the sub-cortical (peripheral) liver is generally considered to be the principal hematopoietic site from early development (Chen and Turpen, 1995; Hadji-Azimi et al., 1987, 1990; Nogawa-Kosaka et al., 2011). However, recent findings, combined with older literature suggest that in fact different amphibian species may actually localize their blood cell development to different organs and tissues (Akulenko, 2012; Brunst, 1958; Carver and Meints, 1977; Durand et al., 2000; Golub et al., 2004; Hadji-Azimi et al., 1987, 1990; Lane and Sheets, 2002). In this regard, it is noteworthy that although the amphibian bone marrow is relatively rudimentary and has been largely overlooked as a potential site of hematopoiesis (Hadji-Azimi et al., 1987, 1990), it appears that certain amphibian species utilize this site for blood cell development (discussed below).

2.1. Urodela

Hematopoiesis in urodela (salamanders and newts) is thought to occur within the liver and spleen of both larvae and adults (Brunst, 1958; Durand et al., 2000; Golub et al., 2004). In contrast, to our knowledge there are currently no published studies implicating the bone marrow of urodeles in blood cell development. Notably, urodelians are invaluable animal models for hematopoiesis research owing to their life-long regenerative capacity, which anurans only possess during a short period before the onset of metamorphosis [reviewed in (Godwin and Rosenthal, 2014)]. Recent work by Lopez et al. (2014) has demonstrated that axolotl spleen cells are very good producers of hematopoietic colony stimulating factors and are a major source of pluripotent hematopoietic stem cells (HSCs). Stimulation of axolotl splenic HSCs with the pokeweed mitogen-stimulated spleen cell medium (PWM-SCM) gives rise to a predominant fraction of erythroid-lineage cells as well as mixed mononuclear and polymorphonuclear myeloid progenitor populations (Lopez et al., 2014). The detection of PWM-SCM-responsive colony forming units strongly suggests that hematopoiesis in axolotls is confined to spleen and liver but absent from bone marrow, thymus, and kidney tissues (Lopez et al., 2014). This is further underlined by the observation that GFP^+ spleen- and liver-derived hematopoietic cells, adoptively transferred into γ-irradiated albino axolotl recipients differentiate only within the liver and spleen tissues (Lopez et al., 2014). Furthermore, when two axolotls are connected by probiosis, thus sharing their circulating HSCs, GFP^+ cells from one animal home into and populate the liver and spleen of the other animal. Strikingly, irradiated but not extraneously reconstituted animals suffer severe anemia, confirming that the liver and spleen indeed serve as sources of hematopoietic precursors and sites of axolotl blood-cell development (Lopez et al., 2014). Although these investigations do not completely rule out the possibility that other axolotl tissues may be contributing to hematopoiesis, they strongly suggest that the reconstituted liver and spleen cell populations are sufficient to circumvent cell depletion resulting from γ-irradiation.

2.2. Anura

The anuran tadpole and adult life stages exhibit distinct physiology and ecological niches that likely include exposure to different potential pathogens. As such it is not surprising that the blood cell development of these life stages is also distinct.

2.2.1. Tadpole hematopoiesis

In *Xenopus,* the embryonic anterior blood island (equivalent to the mammalian yolk sac) that forms soon after neurulation (NF stage 20, 22–23 h post-fertilization) serves as the initial source of myeloid cells, whereas erythroid and lymphoid blood cell lineages originate from the adjacent posterior ventral blood island (Ciau-Uitz et al., 2010b; Kau and Turpen, 1983; Maeno et al., 1985). At this early developmental stage (NF stage 30), primitive myeloid cells called 'myelocytes' comprise the initial blood cells to differentiate within the *Xenopus* embryo and can be seen migrating throughout the developing organism (Costa et al., 2008). Even before development of functional vasculature, these myelocytes are readily recruited to sites of injury or pathogenic challenge, possibly serving as transient innate immune effector cells. The long-term functional roles, modes of action, and ultimate fates of myelocytes during later animal life remain to be defined.

Early on during *Xenopus* larval development (NF stage 40–46) and subsequent to the establishment of blood circulation, the liver becomes the primary hematopoietic site, responsible for the formation of erythrocytes, leukocytes and B cells (Chen and Turpen, 1995). At this early developmental stage, when the spleen is still absent, expression of a fluorescence marker under the control of cell type-specific promoters in transgenic animals has revealed that these larvae possess complex and diverse populations of myeloid cells including granulocytic and monocytic lineages (Paredes et al., 2015).

Based on *Xenopus* studies, the sub-cortical (peripheral) liver is generally considered to be the principal anuran hematopoietic site from early development (Chen and Turpen, 1995; Nogawa-Kosaka et al., 2011). However, hematopoiesis in tadpoles of the European common frog, *Rana temporaria,* appears to be restricted to pronephric interstices of kidneys, and does not occur in liver at all (Meseguer et al., 1985). Similarly, tadpoles of the edible common frog *Rana esculenta* employ their trunk pronephrous tissue for granulopoiesis (Frank, 1988, 1989). Interestingly, during embryogenesis and early larval development of the related leopard frog, *Rana pipiens*, the pronephrous tissue is also involved in hematopoiesis, although myeloid, lymphoid and erythroid lineages are also generated in the liver (Carpenter and Turpen, 1979). More specifically, precursors of these lineages are localized in the endothelia-lined sinusoids found within the sub-capsular liver as well as deeper within the hepatic tissues (Turpen et al., 1979). The respective contribution of kidney and liver to *R. pipiens* hematopoiesis is presently not clear. It is also notable that, as described below, the bone marrow of post-metamorphic *Rana spp.* may serve as an important site of hematopoiesis (Carver and Meints, 1977). We believe that it would be useful to reexamine some of this older work, using more advanced molecular techniques to delineate the exact contributions of respective *R. spp.* tissues to the development of distinct blood cell lineages at distinct developmental stages.

2.2.2. Adult hematopoiesis

Since urodelians appear to lack bone marrow-mediated hematopoiesis, anurans may represent the first vertebrate phylogenetic group to involve bone marrow in blood cell development. Immunohistological studies of the adult American bullfrog (*Rana catesbeianus*) have revealed the presence of active hematopoiesis within the vertebrae, femur and finger bone marrow as well as in