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Amphibian myelopoiesis

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A B S T R A C T

Macrophage-lineage cells are indispensable to immunity and physiology of all vertebrates. Amongst these, amphibians represent a key stage in vertebrate evolution and are facing decimating population declines and extinctions, in large part due to emerging infectious agents. While recent studies indicate that macrophages and related innate immune cells are critically involved during these infections, much remains unknown regarding the ontogeny and functional differentiation of these cell types in amphibians. Accordingly, in this review we coalesce what has been established to date about amphibian blood cell development (hematopoiesis), the development of key amphibian innate immune cells (myelopoiesis) and the differentiation of amphibian macrophage subsets (monopoiesis). We explore the current understanding of designated sites of larval and adult hematopoiesis across distinct amphibian species and consider what mechanisms may lend to these species-specific adaptations. We discern the identified molecular mechanisms governing the functional differentiation of disparate amphibian (chiefly *Xenopus laevis*) macrophage subsets and describe what is known about the roles of these subsets during amphibian infections with intracellular pathogens. Macrophage lineage cells are at the heart of so many vertebrate physiological processes. Thus, garnering greater understanding of the mechanisms responsible for the ontogeny and functionality of these cells in amphibians will lend to a more comprehensive view of vertebrate evolution.

1. Introduction

Amphibians have become popular animal models for comparative and developmental studies of immunity, reviewed in (Du Pasquier et al., 1989; O'Rourke, 2007; Robert and Ohta, 2009). Of the more commonly studied frog genera including *Xenopus*, *Rana*, *Hyla* and *Bufo*, the African clawed frog species, *X. laevis* and *X. tropicalis* remain by far the primary models for research, owing to their sequenced genomes and availability of reagents (de Abreu Manso et al., 2009; Maniatis and Ingram, 1971; Manning and Horton, 1982; Robert and Ohta, 2009). Moreover, the immunologically distinct tadpole and adult amphibian life stages, separated by drastic tissue remodeling during metamorphosis, add to the appeal of amphibian species as research models of the ontogeny of immune systems (O'Rourke, 2007).

While the overall cellular and genetic composition of the amphibian immune system bears a fundamental resemblance to those of mammals, reviewed in (Flajnik, 2018), there are also notable distinctions. For instance, while the bone marrow is the major site for hematopoiesis in mammals, the liver periphery and spleen tissues are considered to be the principal hematopoietic sites in many post-metamorphic amphibian species (Chen and Turpen, 1995; Golub et al., 2004; Nogawa-Kosaka et al., 2011). With the exception of the spleen, amphibians also lack secondary lymphoid organs. Lymphocytes accumulate in the liver periphery, kidney and along the intestines, but do not form organized lymph nodes, reviewed in (Du Pasquier et al., 1989; Robert and Ohta, 2009).

Amphibian populations are experiencing an alarming global decline as the result of complicated factors such as habitat destruction, temperature changes and pollution, concomitant with elevated emerging bacterial, fungal, and viral infections, reviewed in (Rollins-Smith, 2017). Presumably, these compounding factors (at least in part) manifest in decreased capacities of amphibians to mount successful immune responses against these pathogens, resulting in the increased animal mortalities. Thus, to curtail these amphibian declines, we must first understand the sources and functional roles of amphibian immune cells and their responses towards infections caused by these pathogens.

1.1. Hematopoiesis

In adult vertebrates, hematopoiesis takes place within designated tissues. While this process occurs in the bone marrow in mammals and birds (Bartelmez et al., 1989; Garceau et al., 2010; Kriegler et al., 1994), it is localized to the head kidney in fish (Belosevic et al., 2006; Neumann et al., 2000). In each of these sites, the pluripotent hematopoietic stem cells (HSCs) give rise to common lymphoid precursors (CLP) or common myeloid precursors (CMP). The CLPs further differentiate to give rise to

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precursors for B and T cells (pre-T/pre-B), and natural killer (NK) cells. Likewise, CMPs give rise to megakaryocyte/erythrocyte precursors (MEP) or granulocyte/macrophage precursors (GMP). These hematopoietic steps (for *X. laevis*) are depicted in Fig. 1A. The sites of amphibian hematopoiesis vary between species and occur in distinct sites including liver periphery, kidney, and the bone marrow, as discussed below.

1.2. Liver-mediated hematopoiesis in amphibians

The amphibian subcapsular/peripheral liver is thought to be the principal hematopoietic organ during the early stages of amphibian development and in some cases into adult life (Chen and Turpen, 1995; Nogawa-Kosaka et al., 2011). In general, urodeles and more primitive anurans such as *Xenopus* appear to utilize the liver periphery towards both larval and adult hematopoiesis, whereas more recently diverged anuran species may employ the liver and/or kidney for tadpole hematopoiesis and their bone marrow for adult blood cell development (Lopez et al., 2014). Moreover, amphibian hepatic hematopoietic activity is subject to seasonal variations. For instance, research on *Pelophylax ridibundus,* has revealed that spring and summer seasons are favorable to granulocyte differentiation with autumn and summer, favorable to erythropoiesis (Akulenko, 2012).

Akin to mammals, resident liver macrophages known as Kupffer cells, have been identified in amphibian livers and have been demonstrated to bear high resemblance to mammalian Kupffer cells in morphology and function (Sichel et al., 2002). However, unlike the mammalian counterparts, these amphibian liver phagocytes possess the ability to produce melanin (Guida et al., 2000; Purrello et al., 2001; Sichel et al., 2002), where the main function of this melanin pigmentation is believed to be the regulation of cytotoxic substances such as ions and free radicals (Gallone et al., 2007; Sichel, 1988). Owing to environmental changes such as temperature fluctuations, food availability, and other seasonal change, the liver undergoes certain morphological changes, and these pigmented Kupffer cells are believed to contribute to these changes (Barni et al., 1999). Notably, amphibian infections with protist Perkinsea pathogen(s) represent the most recently recognized threat to amphibian global diversity (Chambouvet et al., 2015, 2020; Isidoro-Ayza et al., 2017). Since Perkinsea target amphibian livers (Isidoro-Ayza et al., 2017), we anticipate that Kupffer cells, which are critical to vertebrate liver integrity (Dou et al., 2019), will be important effectors in the immune responses against this emerging pathogen.

1.3. Kidney-mediated hematopoiesis in amphibians

While many studied amphibians are thought to utilize their livers as principal sites of larval hematopoiesis, other tadpole amphibians employ their kidney as their site of blood cell development. For example, tadpoles of the European common frog, *Rana temporaria* utilize the pronephric interstices of their kidneys for hematopoiesis and do not appear to use their livers at all towards these processes (Meseguer et al., 1985). Likewise, the tadpoles of the edible common frog, *Rana esculenta* use their trunk kidney tissue for granulocyte development, and possibly other forms of hematopoiesis (Frank, 1988, 1989). During embryogenesis and early tadpole development of the leopard frog, *Rana pipiens* the kidney is used towards granulopoiesis and some erythropoiesis (Carpenter and Turpen, 1979). While Carpenter and Turpen (1979) observed almost no lymphocyte development in larval *R. pipiens* kidneys, Zettergren reported that these leopard frog tadpoles do use their kidneys for B cell development (Zettergren, 1982). Notably, whereas the former report relied primarily on histological observation, the latter used the much more objective approach of immunohistochemistry. Interestingly, the adult American bullfrog *Lithobates catesbeianus* utilizes both bone marrow and kidney towards the production of granulocytes, monocytes, erythrocytes, and lymphocytes (de Abreu Manso et al., 2009). With all these observed differences, it will be invaluable to determine the precise and respective contributions of the kidney and liver to amphibian hematopoiesis of disparate life stages of distinct amphibian species.

1.4. Bone marrow-mediated hematopoiesis in amphibians

In mammals, the bone marrow is a soft, fatty tissue that is the principal site of adult hematopoiesis. In contrast, different amphibian species exhibit the use of distinct organs and tissues towards blood cell development (Akulenko, 2012; Carver and Meints, 1977; Golub et al., 2004; Lane and Sheets, 2002). Some of the amphibian species use their bone marrow towards hematopoiesis, thus representing the earliest evolutionary origins of this site for blood cell development. For instance, in *Rana ridibunda,* the bone marrow serves as the designated site for hematopoiesis and appears to be subjected to seasonal fluctuations (Akulenko, 2012). Research on *Rana* (*Lithobates*) *catesbeianus* has demonstrated active hematopoiesis in the bone marrow of the vertebrae, femur and fingers, but no blood cell development was found to take place in the liver or spleen tissues (de Abreu Manso et al., 2009). Morphological analysis of erythroid cells in *R. catesbeianus* adults revealed that the erythroid-lineage cells were found exclusively in the livers but not in the bone marrow of these adult frogs (Maniatis and Ingram, 1971). However, it is difficult to deduce the hematopoietic site in this species, as the results are based solely on the cells containing hemoglobin. It is possible that these cells may originate at distinct hematopoietic site(s) and migrate to the bone marrow following metamorphosis (Maniatis and Ingram, 1971).

While the liver periphery and spleen tissues were thought to be the principal sites of hematopoiesis in adult *X. laevis* (Hadji-Azimi et al., 1987; Lane and Sheets, 2002), our work demonstrated that the development of macrophage, granulocyte and at least some dendritic cell-lineages occur in the bone marrow of these animals (Hossainey et al., 2023; Yaparla et al., 2019) Fig. 1). Notably, other leukocyte precursors such as B cells are clearly derived within the *X. laevis* liver periphery and spleen tissues and not in the bone marrow (Hadji-Azimi et al., 1990; Marr et al., 2007).

As demonstrated in well-characterized mammalian species, myelopoiesis (*X. laevis* myelopoiesis depicted in Fig. 1A&C) is the branch of hematopoiesis through which common myeloid progenitor (CMP) cells give rise to granulocyte/macrophage progenitors (GMPs) or megakaryocyte/erythrocyte progenitors (MEP (Ciau-Uitz et al., 2014; Cumano and Godin, 2007; Orkin and Zon, 2008)). In turn, GMPs differentiate into either granulocyte (Gran)- or macrophage (Mφ)-lineage cells in response to lineage-specific growth factors (De Kleer et al., 2014). With these progressions through lineage commitment steps, GMP lose their Gran-producing capacities, becoming monocyte/macrophage and dendritic cell progenitors (MDP). Subsequently, MDP give rise to either common monocyte progenitors (cMoP) that are precursors to extensive repertoires of morphologically and functionally distinct macrophage subsets (Naik et al., 2006) or to common dendritic cell (DC) precursor (CDP). Distinct macrophage subsets acquire their respective functional states upon exposure to tissue- and/or immune response-specific factors. However, and as described in greater detail below, all macrophage development requires signaling through the colony stimulation factor-1 receptor (CSF-1R, *i.e.*, macrophage colony-stimulating factor receptor; M-CSFR) on the surface of GMP and CDP (Lin et al., 2019). Signaling through the CSF-1R is elicited by two ligands, CSF-1 (*i.e.,* M-CSF) and interleukin-34 (IL34) (Boulakirba et al., 2018; Chihara et al., 2010), which culminates in macrophage differentiation and functionality (Belosevic et al., 2006; Bonifer and Hume, 2008; Jones and Ricardo, 2013). Our recent work indicates that cells in the *X. laevis* bone marrow have the potential for differentiating into granulocytes, distinct macrophage subsets or into dendritic cells in response to cognate granulocyte- (CSF-3), macrophage- (CSF-1/IL-34) or dendritic cell- (fms-like tyrosine kinsase-3 ligand, FLT3L) specific growth factors (Hossainey et al., 2023) Fig. 1A). This suggests that if the *X. laevis* myelopoiesis follows down the same sequential differentiation pathways as those characterized in

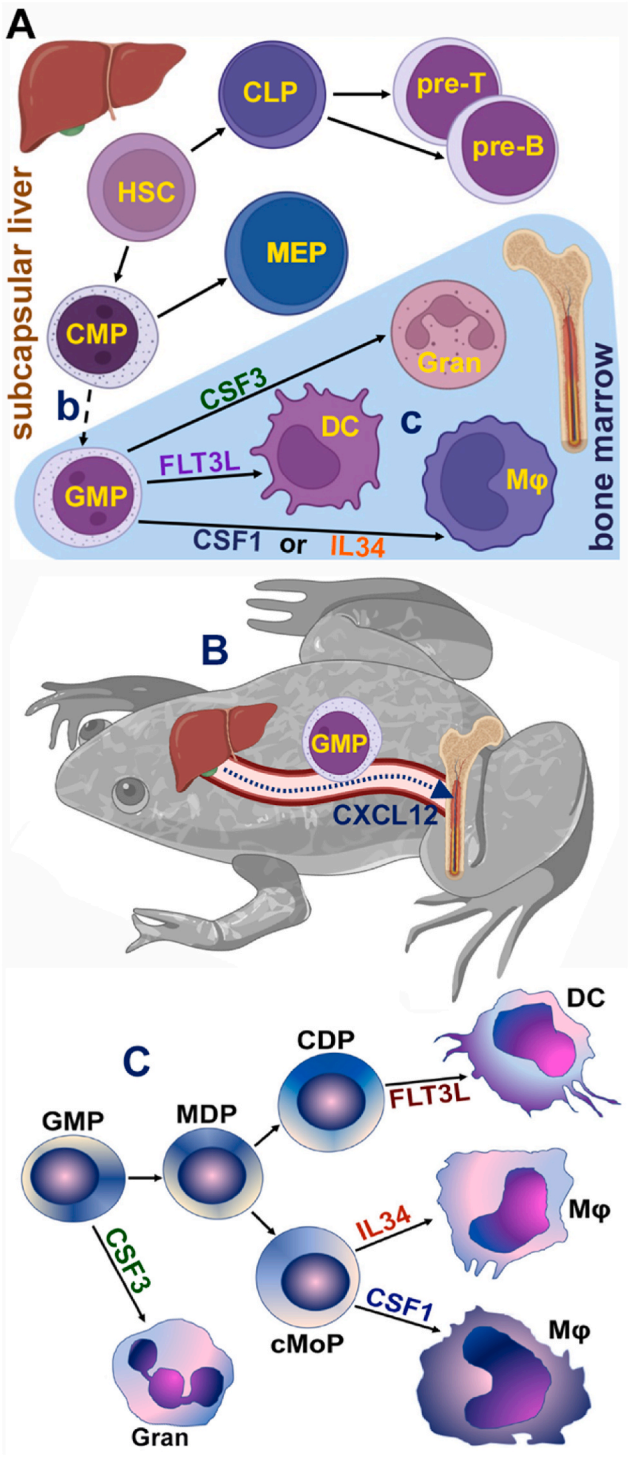

Fig. 1. *Xenopus laevis* segregates myelopoiesis away from general hematopoiesis. (**A**) *X. laevis* pluripotent, lymphoid and some myeloid progenitors are located in the subcapsular (peripheral) liver whereas granulocyte/macrophage precursors are found in the bone marrow of these animals. (**B**) Granulocyte (Gran)/macrophage (Mφ) precursors (GMP) are trafficked/homed to the *X. laevis* bone marrow by the CXCL12 chemokine. (**C**) Once in the bone marrow, GMP are differentiated into distinct myeloid subsets via stimulation with lineage-specific cytokines/growth factors. *Abbreviations*: CDP-common dendritic cell (DC) precursor; CLP-common lymphoid progenitor; cMoP-common monocyte/macrophage precursor; CMP-common myeloid progenitor; CSF-colony-stimulating factor; FLT3L-fms-like tyrosine kinase 3 ligand; GMP-granulocyte/macrophage precursor; HSC-hematopoietic stem cell; IL34-interleukin-34; MDP-monocyte/dendritic cell (DC) precursor; MEP-megakaryocyte/erythrocyte precursor. Images in panels A and B were generated via BioRender.

mammals, then the bone marrow of these animals contains GMP, MDP, cMoP, and CDP (Fig. 1C).

Some amphibian species, such as those belonging to the genus *Rana,* additionally utilize their bone marrow as a site for erythropoiesis (Carver and Meints, 1977). It is noteworthy that amphibians that utilize their bone marrow for hematopoiesis tend to be more terrestrial. As an example, studies of bone marrow vasculature in urodelian and anuran species have revealed that the appearance of fat in the bone marrow is unrelated to its involvement as a hematopoietic site (Tanaka, 1976). In contrast and akin to the mammalian bone marrow, which consists of sub-endosteal veins that are used for draining blood, more terrestrial amphibian species appear to have similar bone marrow vasculature concomitant with more developed bone marrow hematopoietic activity (Tanaka, 1976). This contrasts the earlier branching amphibian lineages such as caecilians (limbless amphibians), urodelians (salamanders/newts) and primitive anurans (frogs/toads; *Bombina* and *Xenopus* genera), which have limited to no bone marrow hematopoietic activity and likewise lack extensive vascular innervation of these sites, reviewed in (Grayfer and Robert, 2016). It has thus been aptly proposed that the evolution and intricacy of bone marrow vasculature is linked to the occurrence and efficacies of hematopoiesis in this tissue (Tanaka, 1976).

1.5. *Monopoiesis in anuran amphibians*

Monopoiesis refers to the development of macrophage-lineage cells, and the commitment of myeloid progenitor cells to this lineage is marked by the surface expression of colony stimulating factor-1 receptor (CSF-1R; Tagoh et al., 2002). As macrophage progenitors develop and undergo differentiation, the magnitude of CSF-1R expression increases, both at transcriptional and protein levels (Bonifer and Hume, 2008; Krysinska et al., 2007; Rosa et al., 2007). The CSF-1R expression is indispensable for macrophage-lineage cell commitment, differentiation and often survival (Cecchini et al., 1994; Stanley et al., 1978). Indeed, recent single-cell sequencing analyses of immune cell populations from seven evolutionarily distinct vertebrate species including fish, frogs, and mammals, underlined CSF1R as the single most important macrophage signaling component across all of these species (Jiao et al., 2023). This contrasts with many other marker and effector genes, which vary depending on the species source of macrophages, presumably coinciding with their unique respective physiological and immune demands.

Prior to commitment (indicated by CSF-1R surface expression), stimulation of HSCs with macrophage growth factor, colony stimulating factor-1 (CSF-1) does not drive the monopoiesis of HSCs, underlining the importance of CSF-1R (Bartelmez et al., 1989; Kriegler et al., 1994). Notably, while liver periphery of *X. laevis* is considered to be the primary site of hematopoiesis (Hadji-Azimi et al., 1987, 1990; Lane and Sheets, 2002), the progenitor cells that are committed to macrophage-lineage appear to reside predominantly, if not exclusively in the bone marrow, away from the liver periphery (Grayfer and Robert, 2013a; Yaparla et al., 2016). Consistent with this notion, upon recombinant (r)CSF-1 stimulation, *X. laevis* bone marrow cells significantly upregulate genes encoding hallmark transcription factors (TFs) associated with macrophage differentiation (Yaparla et al., 2020). In contrast, cells derived from *X. laevis* liver periphery possess greater baseline expression of TFs associated with HSCs, erythroid, and lymphoid cell differentiation, compared to the bone marrow (Yaparla et al., 2020). Based on these findings, we anticipate that while the final stages of macrophage and granulocyte development occur within the *Xenopus* bone marrow, the precursor populations to these cells arise from the hematopoietic liver periphery and/or spleen tissues. In support of this hypothesis, we recently demonstrated that such progenitors are produced in the liver periphery and likely undergo migration/homing to the bone marrow

through blood circulation in response to the CXCL12 chemokine, produced by the bone marrow (Yaparla et al., 2020; Fig. 1B). This is consistent with what occurs in mammals, wherein CXCL12 is responsible for homing HSCs to the bone marrow (Hill et al., 2004; Khurana et al., 2014). It is intriguing that this strategy for homing/retaining macrophage progenitors has been evolutionarily conserved in *X. laevis*. Notably, HSC migration and homing in mammals is influenced by a variety of additional factors such as adhesion molecules, activation/signaling molecules and extracellular calcium concentration in the bone marrow (Ciriza et al., 2012; Ciriza and García-Ojeda, 2010; Lam et al., 2011; Potocnik et al., 2000; Smith-Berdan et al., 2011). Likely, several of these factors are also involved in facilitating retention and further differentiation of GMPs in the *X. laevis* bone marrow.

1.6. Colony stimulating factor-1 and vertebrate macrophages differentiation

As described above, colony stimulating factor-1 (CSF-1, M-CSF) activates CSF-1R, expressed on macrophage precursors and derivative phagocyte populations (Guilbert and Stanley, 1980; Lichanska et al., 1999). In birds and mammals, single, alternatively spliced mRNA codes for membrane-bound and secretory forms of CSF-1 (Manos, 1988; Rettenmier and Roussel, 1988). The N-terminal region (150 amino acids) of the two forms of CSF-1 in mammals is sufficient for mediating the biological activity of these cytokines and is not affected by splicing (Koths, 1997; Taylor et al., 1994). Teleost fish express two CSF-1 genes on distinct chromosomes that are believed not to undergo the extensive alternative splicing seen in the mammalian counterparts (Wang et al., 2008). Whether the gene products encoded by these two teleost CSF-1 genes are similar to mammalian CSF-1 splice variants in functionality remains to be explored. *Xenoponidae* amphibians possess a single CSF-1 gene (Grayfer and Robert, 2013b, 2015) that undergoes alternative splicing.

Xenopus CSF-1R includes five putative immunoglobulin domains, structurally conserved cysteine residues, and a disrupted tyrosine kinase domain (Grayfer et al., 2014). Although the protein sequences of CSF-1R across vertebrates are poorly conserved, especially in the region that codes for the extracellular domain, the trademark features such as those mentioned above seem to be evolutionary conserved. The amino acid sequence of the respective CSF-1 ligands among vertebrates also display poor sequence identity (Grayfer et al., 2014), which presumably reflects the selective pressure to enable the cognate ligand-receptor binding.

1.7. Interluekin-34 and vertebrate macrophage differentiation

In addition to CSF-1, CSF-1R is also activated by an alternative ligand, interleukin-34 (IL-34), which contributes to monopoiesis (Lin et al., 2008). Although both CSF-1 and IL-34 share the same receptor, these cytokines exhibit distinct tissue-specific gene expression patterns, suggesting that they are involved in disparate biological functions (Boulakirba et al., 2018; Ge et al., 2019). Immunomodulatory effects of IL-34 have also been characterized in autoimmune diseases such as rheumatoid arthritis (RA), a condition which is associated with bone destruction (Chemel et al., 2012). Macrophages are the central immune cells in RA pathogenesis, and IL-34 facilitates the infiltration and differentiation of these cells and enhances the production of proinflammatory cytokines (Chemel et al., 2012), suggesting that IL-34 has a proinflammatory function. IL-34-deficient mice display a substantial reduction in microglia and Langerhans cells, underlining the importance of this cytokine in the differentiation of these cell types (Greter et al., 2012; Wang and Colonna, 2014). Remarkably, IL-34 may also be involved in anti-inflammatory contexts, whereby IL-34 produced by T regulatory cells contributes to transplant tolerance (Bézie et al., 2015).

Under pathological conditions, IL-34 contributes to the pathogenesis of various diseases such as neurological, inflammatory, transplant rejection, and infections by exerting its effects on a variety of cell types including regulatory T cells, osteoclasts and fibroblasts (Ge et al., 2019). Our recent work indicates that the *X. laevis* IL-34 is essential for the development of a subset(s) of macrophages that protect frogs from Frog Virus 3 (FV3; Grayfer and Robert, 2015, 2014; Yaparla et al., 2018) and mycobacteria (Popovic et al., 2019) infections. Moreover, we now know that these antiviral and anti-mycobacterial properties of IL-34-derived macrophages are conserved in humans (Paquin-Proulx et al., 2018; Popovic et al., 2019).

IL-34 also binds to receptor protein-tyrosine phosphatase zeta (PTP-ζ) and syndecan-1, which are expressed on neural progenitors, glial cells and glioblastoma cells, indicating that this cytokine plays additional roles beyond monopoiesis (Nandi et al., 2013). PTP-ζ is not expressed by mammalian or frog macrophages, and to our knowledge, there are no reports of involvement of syndecan-1 in myelopoiesis.

1.8. Xenopus laevis CSF-1- and IL-34-macrophages

While the biological necessity of two macrophage growth factors remains to be fully understood, *X. laevis* macrophage subsets derived from recombinant (r)CSF-1 and rIL-34 (CSF-1-macrophages and IL-34-macrophages, respectively) are functionally distinct (Grayfer and Robert, 2014, 2015; Yaparla et al., 2018). This is underscored by the fact that these macrophage subsets not only bear striking morphological differences (Fig. 2A), but they also express vastly different gene repertoires, including distinct proportions and types of genes involved in immune and non-immune processes (Fig. 2B). In line with these transcriptional differences, the frog CSF-1-Mφs are more phagocytic and display significantly more robust bactericidal activity upon challenge with *Escherichia coli*, in comparison to IL34-macrophages (Grayfer and Robert, 2015). Compared to CSF-1-macrophages, the frog IL-34-macrophages possess considerably greater capacity to generate reactive oxygen species (ROS) and accordingly, exhibit greater transcript levels of genes that encode NADPH oxidase enzyme (Grayfer and Robert, 2015). Moreover, these differences are supported by *in vivo* studies (Grayfer and Robert, 2015), underlining the functional differences of these macrophage subsets.

1.9. The roles of amphibian macrophages during intracellular infections

1.9.1. Frog Virus 3

Infections by Frog Virus 3 (FV3; family Iridoviridae) and other members of the Ranavirus genus represent a serious concern to global amphibian populations (Bayley et al., 2013; Chinchar, 2002; Gray et al., 2009). While the precise FV3 cell tropisms remains to be fully defined, amphibian macrophages appear to be critical not only to antiviral defenses against this pathogen but also to the FV3 infection strategies (Grayfer and Robert, 2015, 2016). Consistent with this notion, *X. laevis* CSF-1- and IL-34-macrophages play very different roles during FV3 infections (Grayfer and Robert, 2014, 2015; Yaparla et al., 2018). While the tadpole and frog CSF-1-macrophages confer susceptibility to FV3 infection, IL-34-macrophages contribute to antiviral resistance against this pathogen (Grayfer and Robert, 2014, 2015; Yaparla et al., 2018). Enriching tadpole IL-34-macrophages before FV3-challenge extends animal survival whereas enriching tadpole CSF-1-macrophages prior to infection, reduces animal survival times (Grayfer and Robert, 2014). Moreover, the heightened antiviral capacities seen in frog IL-34-macrophages reflect their robust gene expression of type I and type III interferon cytokines as well as genes that encode for antiviral restriction factors (Yaparla et al., 2018). We also found that akin to frog CSF-1-/IL-34-macrophage antiviral dichotomy, human CSF-1-macrophages are significantly more susceptible to HIV-1 than human IL-34-macrophages (Paquin-Proulx et al., 2018). Supporting this role of the human IL-34, this cytokine has also been shown to inhibit Hepatitis B Virus replication in humans, both *in vivo* and *in vitro*, although the underlying mechanisms behind this inhibition are currently unknown (Cheng et al., 2017).

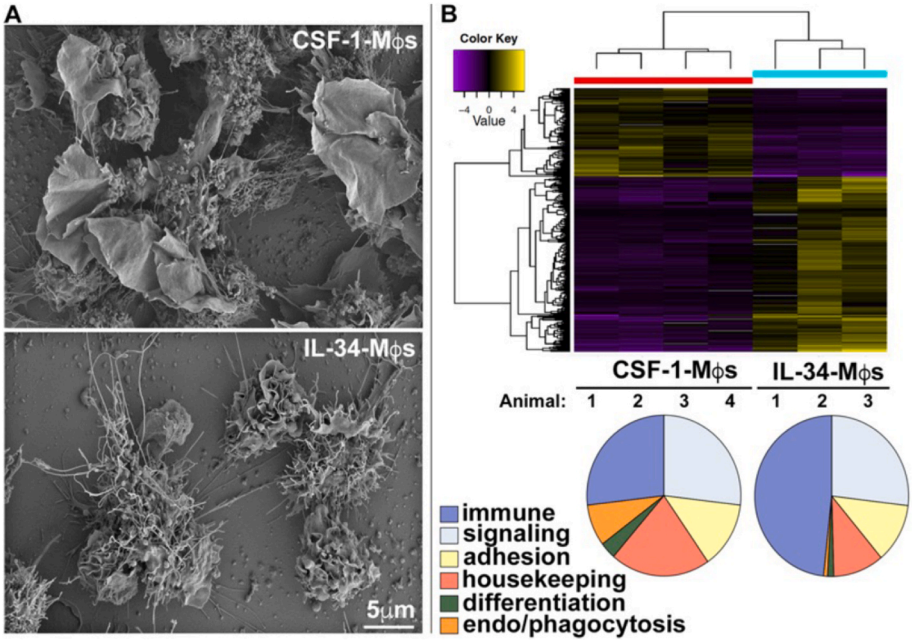

Fig. 2. *X. laevis* macrophages differentiated by colony-stimulating factor-1 and interleukin-34 (CSF-1- and IL-34-Mφs, respectively) are morphologically and transcriptionally distinct. (**A**) Scanning electron microscopy images of *X. laevis* CSF-1- and IL-34-Mφs. (**B**) Next Gen RNA sequencing analysis of *X. laevis* CSF-1- and IL-34-Mφs indicates that these cells possess drastically different immune and non-immune gene expression profiles. These differences include distinct proportions and types of genes (depicted by inset pie charts in B) involved in processes such as immune responses, cell adhesion and signaling, endocytosis and phagocytosis as well as housekeeping genes.

Converging studies indicate that although anuran amphibians clear primary FV3 infections, residual virus persists within the frog kidneys and myeloid populations (Robert et al., 2007, 2014; Samanta et al., 2021), thus presumably serving as reservoir sources of environmental dissemination. Notably, the transcriptional status of the persisting FV3 has not been well defined, with questions regarding whether this virus becomes quiescent or maintains an active transcriptional state during such prolonged/chronic infections. In this regard, our findings indicate that at least in juvenile froglets infected with FV3, the virus remains transcriptionally active and infectious, contesting the idea of quiescence/latency (Hossainey et al., 2021). It appears that FV3-infected frog CSF-1-macrophages result in more prominent kidney FV3 infections in both tadpoles and adult frogs and culminate in more substantial reservoirs of lingering, transcriptionally active and infectious FV3 in post-metamorphic animals. These infections are marked by infiltrating leukocytes, fibrosis, and an overall immunosuppressive state. Conversely, we observed that the antiviral effects of IL-34-macrophages are short-lived and are lost as FV3 infections progress. Further studies across different developmental stages of disparate frog species are needed to reconcile whether and under what immune/physiological contexts FV3 and other ranaviruses might become quiescent.

1.9.2. Mycobacteria

The causative agent of human tuberculosis (TB), *Mycobacterium tuberculosis* (*Mtb*) continues to be a major cause of death by an infectious agent around the globe (WHO, 2016.). A key feature of mycobacterial disease is the formation of granulomas around mycobacteria-infected macrophages, surrounded by infiltrating uninfected macrophages, foamy macrophages, multi-nucleated cells, epithelial cells, T cells and fibroblasts (Cosma et al., 2004; Ramakrishnan et al., 2000). These granuloma-enclosed mycobacteria remain dormant as latent infections, which may reactivate in response to stress or injury, leading to active infectious TB months to years after initial infections (Bouley et al., 2001). Unfortunately, a lack of animal models that permit combined *in vitro* and *in vivo* studies of *Mtb* has been a major barrier to research in this field. In turn, the causative agent of aquatic vertebrate TB, *Mycobacterium marinum* (*Mm*) has gained prominence as an alternative/complementary TB model, granting many new insights into mycobacteria-host interactions (Berg and Ramakrishnan, 2012; Bouley et al., 2001; Ramakrishnan, 2013), owing to its close genetic relatedness to *Mtb* and the availability of alternative, natural host aquatic animal models.

We recently adopted the *Xenopus laevis* frog-*Mm* surrogate infection model to study host susceptibility and resistance to mycobacteria. Using a combination of *in vitro* and *in vivo* studies, we showed that adult *X. laevis* CSF-1-macrophages exacerbate *Mm* infections, are more susceptible to infiltration by mycobacteria and possess less robust mycobactericidal capacities than frog IL-34-macrophages (Popovic et al., 2019). We linked this susceptibility to greater CSF-1-macrophage expression of receptors used for mycobacterial entry as well as greater capacity of *Mm* to escape phago-lysosomal killing once inside CSF-1-macrophages. Conversely, frog IL-34-macrophages exhibit greater resistance to mycobacteria *in vivo*, are less susceptible to *Mm* entry and more effectively eliminate internalized mycobacteria, at least in part through phago-lysosomal destruction. Moreover, adult frogs enriched for IL-34-macrophages and challenged with heat-killed *Mm* possessed significantly lower mycobacterial burdens than control or CSF-1-macrophage-enriched frogs (Hossainey et al., 2023) upon rechallenge with viable *Mm*, suggesting that frog IL-34-macrophages may also facilitate better priming of these animals' adaptive immune responses. This is consistent with our observation that frog IL-34-macrophages express greater levels of surface MHC class I, are better at eliciting mixed leukocyte responses and are much more transcriptionally similar to frog dendritic cells than CSF-1-macrophages (Hossainey et al., 2023). Akin to what appears to be an evolutionarily conserved CSF-1-/IL-34-macrophage antiviral dichotomy, we also found that human CSF-1-macrophages are likewise much more permissive to mycobacterial entry and spread than counterpart human IL-34-macrophages (Popovic et al., 2019).

Possibly all vertebrate CSF-1-macrophages are more prone to intracellular macrophage-tropic pathogens than IL-34-macrophages, although we anticipate that tissue microenvironments play additional critical roles in how these macrophage types respond to pathogens *in vivo*. Indeed, we showed that both CSF-1- and IL-34-macrophages may be counter-polarized by IL-34 and CSF-1 to be respectively less and more susceptible to pathogens like *Mm* and FV3 (Popovic et al., 2019; Yaparla et al., 2018). We found that these frog macrophage subsets possess disparate pathogen recognition capacities, while stimulation with certain microbe-associated molecular patterns renders CSF-1-macrophages more resistant to FV3 and IL-34-macrophages more

susceptible to this pathogen (Yaparla et al., 2020). It stands to reason that other cytokines, activation factors and immune stimuli within local tissue milieu may have similar consequences on the functionality of CSF-1- and IL-34-differentiated macrophages.

1.9.3. Concluding remarks

Macrophage-lineage cells encompass a vast array of tissue specific cell types such as skin-resident Langerhans cells, brain microglia, connective tissue histiocytes, bone osteoclasts, liver Kupffer cells and alveolar macrophages, amongst many others. And while we tend to think of macrophages as cells of the immune system, the biological roles played by these cell types far exceed their capacities as immune purveyors. Every vertebrate tissue has its own designated macrophage type (s), ready to respond to whatever derivation from homeostasis, towards returning that tissue back to its designated function.

Our world is inhabited by remarkable spectrum of evolutionary diverged vertebrate species, occupying diverse environments, with their physiologies adapted to those landscapes. In turn, each of those animals' physiologies is comprised of many diverse and specialized tissues, each relying on their own macrophage subset(s) to maintain the integrity of those tissues. One could argue that macrophages are what all vertebrates have in common and thus, by studying the divergent and converged aspects of macrophage biology across these species, we move closer to understanding what unifies and sets apart all vertebrate life on Earth.

Data availability

Data will be made available on request.

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References

Akulenko, N., 2012. Haemopoietic System of the Anurans: the role of bone marrow and liver. Vestn. Zool. 46, e–28.

Barni, S., Bertone, V., Croce, A.C., Bottiroli, G., Bernini, F., Gerzeli, G., 1999. Increase in liver pigmentation during natural hibernation in some amphibians. J. Anat. 195, 19–25.

Bartelmez, S.H., Bradley, T.R., Bertoncello, I., Mochizuki, D.Y., Tushinski, R.J., Stanley, E.R., Hapel, A.J., Young, I.G., Kriegler, A.B., Hodgson, G.S., 1989. Interleukin 1 plus interleukin 3 plus colony-stimulating factor 1 are essential for clonal proliferation of primitive myeloid bone marrow cells. Exp. Hematol. 17, 240–245.

Bayley, A.E., Hill, B.J., Feist, S.W., 2013. Susceptibility of the European common frog *Rana temporaria* to a panel of ranavirus isolates from fish and amphibian hosts. Dis. Aquat. Org. 103, 171–183.

Belosevic, M., Hanington, P.C., Barreda, D.R., 2006. Development of goldfish macrophages in vitro. Fish Shellfish Immunol. 20, 152–171.

Berg, R.D., Ramakrishnan, L., 2012. Insights into tuberculosis from the zebrafish model. Trends Mol. Med. 18, 689–690.

Bézie, S., Picarda, E., Ossart, J., Tesson, L., Usal, C., Renaudin, K., Anegon, I., Guillonneau, C., 2015. IL-34 is a Treg-specific cytokine and mediates transplant tolerance. J. Clin. Invest. 125, 3952–3964.

Bonifer, C., Hume, D.A., 2008. The transcriptional regulation of the *colony stimulating factor-1 receptor* (*csf1r*) gene during hematopoiesis. Front. Biosci. J. Virtual Libr. 13, 549–560.

Boulakirba, S., Pfeifer, A., Mhaidly, R., Obba, S., Goulard, M., Schmitt, T., Chaintreuil, P., Calleja, A., Furstoss, N., Orange, F., Lacas-Gervais, S., Boyer, L., Marchetti, S., Verhoeyen, E., Luciano, F., Robert, G., Auberger, P., Jacquel, A., 2018. IL-34 and CSF-1 display an equivalent macrophage differentiation ability but a different polarization potential. Sci. Rep. 8.

Bouley, D.M., Ghori, N., Mercer, K.L., Falkow, S., Ramakrishnan, L., 2001. Dynamic nature of host-pathogen interactions in Mycobacterium marinum granulomas. Infect. Immun. 69, 7820–7831.

Carpenter, K.L., Turpen, J.B., 1979. Experimental studies on hemopoiesis in the pronephros of Rana pipiens. Different. Res. Biol. Divers. 14, 167–174.

Carver, F.J., Meints, R.H., 1977. Studies of the development of frog hemopoietic tissue *in vitro*. I. Spleen culture assay of an erythropoietic factor in anemic frog blood. J. Exp. Zool. 201, 37–46.

Cecchini, M.G., Dominguez, M.G., Mocci, S., Wetterwald, A., Felix, R., Fleisch, H., Chisholm, O., Hofstetter, W., Pollard, J.W., Stanley, E.R., 1994. Role of colony stimulating factor-1 in the establishment and regulation of tissue macrophages during postnatal development of the mouse. Dev. Camb. Engl. 120, 1357–1372.

Chambouvet, A., Gower, D.J., Jirku, M., Yabsley, M.J., Davis, A.K., Leonard, G., Maguire, F., Doherty-Bone, T.M., Bittencourt-Silva, G.B., Wilkinson, M., Richards, T. A., 2015. Cryptic infection of a broad taxonomic and geographic diversity of tadpoles by Perkinsea protists. Proc. Natl. Acad. Sci. U. S. A. 112, E4743–E4751.

Chambouvet, A., Smilansky, V., Jirku, M., Isidoro-Ayza, M., Itoiz, S., Derelle, E., Monier, A., Gower, D.J., Wilkinson, M., Yabsley, M.J., Lukes, J., Richards, T.A., 2020. Diverse alveolate infections of tadpoles, a new threat to frogs? PLoS Pathog. 16, e1008107.

Chemel, M., Le Goff, B., Brion, R., Cozic, C., Berreur, M., Amiaud, J., Bougras, G., Touchais, S., Blanchard, F., Heymann, M.F., Berthelot, J.M., Verrecchia, F., Heymann, D., 2012. Interleukin 34 expression is associated with synovitis severity in rheumatoid arthritis patients. Ann. Rheum. Dis. 71, 150–154.

Chen, X.D., Turpen, J.B., 1995. Intraembryonic origin of hepatic hematopoiesis in *Xenopus laevis*. J. Immunol. Baltim. Md 1950 154, 2557–2567.

Cheng, S.-T., Tang, H., Ren, J.-H., Chen, X., Huang, A.-L., Chen, J., 2017. Interleukin-34 inhibits hepatitis B virus replication *in vitro* and *in vivo*. PLoS One 12. https://doi.org/10.1371/journal.pone.0179605.

Chihara, T., Suzu, S., Hassan, R., Chutiwitoonchai, N., Hiyoshi, M., Motoyoshi, K., Kimura, F., Okada, S., 2010. IL-34 and M-CSF share the receptor Fms but are not identical in biological activity and signal activation. Cell Death Differ. 17, 1917–1927.

Chinchar, V.G., 2002. Ranaviruses (family *Iridoviridae*): emerging cold-blooded killers. Arch. Virol. 147, 447–470.

Ciau-Uitz, A., Monteiro, R., Kirmizitas, A., Patient, R., 2014. Developmental hematopoiesis: ontogeny, genetic programming and conservation. Exp. Hematol. 42, 669–683.

Ciriza, J., García-Ojeda, M.E., 2010. Expression of migration-related genes is progressively upregulated in murine Lineage-Sca-1+c-Kit+ population from the fetal to adult stages of development. Stem Cell Res. Ther. 1, 14.

Ciriza, J., Hall, D., Lu, A., Sena, J.R.D., Al-Kuhlani, M., García-Ojeda, M.E., 2012. Single-cell analysis of murine long-term hematopoietic stem cells reveals distinct patterns of gene expression during fetal migration. PLoS One 7, e30542.

Cosma, C.L., Humbert, O., Ramakrishnan, L., 2004. Superinfecting mycobacteria home to established tuberculous granulomas. Nat. Immunol. 5, 828–835.

Cumano, A., Godin, I., 2007. Ontogeny of the hematopoietic system. Annu. Rev. Immunol. 25, 745–785.

de Abreu Manso, P.P., de Brito-Gitirana, L., Pelajo-Machado, M., 2009. Localization of hematopoietic cells in the bullfrog (*Lithobates catesbeianus*). Cell Tissue Res. 337, 301–312.

De Kleer, I., Willems, F., Lambrecht, B., Goriely, S., 2014. Ontogeny of myeloid cells. Front. Immunol. 5, 423.

Dou, L., Shi, X., He, X., Gao, Y., 2019. Macrophage phenotype and function in liver disorder. Front. Immunol. 10, 3112.

Du Pasquier, L., Schwager, J., Flajnik, M.F., 1989. The immune system of *Xenopus*. Annu. Rev. Immunol. 7, 251–275.

Flajnik, M.F., 2018. A cold-blooded view of adaptive immunity. Nat. Rev. Immunol. 18, 438–453.

Frank, G., 1988. Granulopoiesis in tadpoles of Rana esculenta. Survey of the organs involved. J. Anat. 160, 59–66.

Frank, G., 1989. Granulopoiesis in tadpoles of Rana esculenta. Ultrastructural observations on the developing granulocytes and on the development of eosinophil granules. J. Anat. 163, 97–105.

Gallone, A., Sagliano, A., Guida, G., Ito, S., Wakamatsu, K., Capozzi, V., Perna, G., Zanna, P., Cicero, R., 2007. The melanogenic system of the liver pigmented macrophages of *Rana esculenta L.* - tyrosinase activity. Histol. 22, 1065–1075.

Garceau, V., Smith, J., Paton, I.R., Davey, M., Fares, M.A., Sester, D.P., Burt, D.W., Hume, D.A., 2010. Pivotal Advance: avian colony-stimulating factor 1 (CSF-1), interleukin-34 (IL-34), and CSF-1 receptor genes and gene products. J. Leukoc. Biol. 87, 753–764.

Ge, Y., Huang, M., Yao, Y.-M., 2019. Immunomodulation of interleukin-34 and its potential significance as a disease biomarker and therapeutic target. Int. J. Biol. Sci. 15, 1835–1845.

Golub, R., André, S., Hassanin, A., Affaticati, P., Larijani, M., Fellah, J.S., 2004. Early expression of two TdT isoforms in the hematopoietic system of the Mexican axolotl. Implications for the evolutionary origin of the N-nucleotide addition. Immunogenetics 56, 204–213.

Gray, M., Miller, D., Hoverman, J., 2009. Ecology and pathology of amphibian ranaviruses. Dis. Aquat. Org. 87, 243–266.
Grayfer, L., Robert, J., 2013a. Colony-stimulating factor-1-responsive macrophage precursors reside in the amphibian *(Xenopus laevis)* bone marrow rather than the hematopoietic subcapsular liver. J. Innate Immun. 5, 531–542.
Grayfer, L., Robert, J., 2013b. Colony-stimulating factor-1-responsive macrophage precursors reside in the amphibian (*Xenopus laevis*) bone marrow rather than the hematopoietic subcapsular liver. J. Innate Immun. 5, 531–542.
Grayfer, L., Robert, J., 2014. Divergent antiviral roles of amphibian (*Xenopus laevis*) macrophages elicited by colony-stimulating factor-1 and interleukin-34. J. Leukoc. Biol. 96, 1143–1153.
Grayfer, L., Robert, J., 2015. Distinct functional roles of amphibian (*Xenopus laevis*) colony-stimulating factor-1- and interleukin-34-derived macrophages. J. Leukoc. Biol. 98, 641–649.
Grayfer, L., Robert, J., 2016. Amphibian macrophage development and antiviral defenses. Dev. Comp. Immunol. 58, 60–67.
Grayfer, L., Edholm, E.-S., Robert, J., 2014. Mechanisms of amphibian macrophage development: characterization of the *Xenopus laevis* colony-stimulating factor-1 receptor. Int. J. Dev. Biol. 58, 757–766.
Greter, M., Lelios, I., Pelczar, P., Hoeffel, G., Price, J., Leboeuf, M., Kündig, T.M., Frei, K., Ginhoux, F., Merad, M., Becher, B., 2012. Stroma-derived interleukin-34 controls the development and maintenance of Langerhans cells and the maintenance of microglia. Immunity 37, 1050–1060.
Guida, G., Gallone, A., Maida, I., Boffoli, D., Cicero, R., 2000. Tyrosinase gene expression in the kupffer cells of Rana esculenta L. Pigment Cell Res. Spons. Eur. Soc. Pigment Cell Res. Int. Pigment Cell Soc. 13, 431–435.
Guilbert, L.J., Stanley, E.R., 1980. Specific interaction of murine colony-stimulating factor with mononuclear phagocytic cells. J. Cell Biol. 85, 153–159.
Hadji-Azimi, I., Coosemans, V., Canicatti, C., 1987. Atlas of adult *Xenopus laevis* hematology. Dev. Comp. Immunol. 11, 807–874.
Hadji-Azimi, Irandokht, Coosemans, V., Canicatti, C., 1990. B-lymphocyte populations in *Xenopus laevis*. Dev. Comp. Immunol. 14, 69–84.
Hill, W.D., Hess, D.C., Martin-Studdard, A., Carothers, J.J., Zheng, J., Hale, D., Maeda, M., Fagan, S.C., Carroll, J.E., Conway, S.J., 2004. SDF-1 (CXCL12) is upregulated in the ischemic penumbra following stroke: association with bone marrow cell homing to injury. J. Neuropathol. Exp. Neurol. 63, 84–96.
Hossainey, M.R.H., Hauser, K., Garvey, C.N., Kalia, N., Garvey, J.N., Grayfer, L., 2023. A perspective into the relationships between amphibian (Xenopus laevis) myeloid cell subsets. Philos. Trans. R. Soc. B (in press).
Hossainey, M.R.H., Yaparla, A., Hauser, K., Moore, T., Grayfer, L., 2021. The roles of amphibian (Xenopus laevis) macrophages during chronic Frog Virus 3 infections. Viruses 13, 2299–2313.
Isidoro-Ayza, M., Lorch, J.M., Grear, D.A., Winzeler, M., Calhoun, D.L., Barichivich, W.J., 2017. Pathogenic lineage of Perkinsea associated with mass mortality of frogs across the United States. Sci. Rep. 7, 10288.
Jiao, A., Zhang, C., Wang, X., Sun, L., Liu, H., Su, Y., Lei, L., Li, W., Ding, R., Ding, C., Dou, M., Tian, P., Sun, C., Yang, X., Zhang, L., Zhang, B., 2023. Single-cell sequencing reveals the evolution of immune molecules across multiple vertebrate species. J. Adv. Res.
Jones, C.V., Ricardo, S.D., 2013. Macrophages and CSF-1: implications for development and beyond. Organogenesis 9, 249–260.
Khurana, S., Melacarne, A., Yadak, R., Schouteden, S., Notelaers, T., Pistoni, M., Maes, C., Verfaillie, C.M., 2014. SMAD signaling regulates CXCL12 expression in the bone marrow niche, affecting homing and mobilization of hematopoietic progenitors. Stem Cell. 32, 3012–3022.
Koths, K., 1997. Structure-function studies on human macrophage colony-stimulating factor (M-CSF). Mol. Reprod. Dev. 46, 31–37 discussion 37-38.
Kriegler, A.B., Verschoor, S.M., Bernardo, D., Bertoncello, I., 1994. The relationship between different high proliferative potential colony-forming cells in mouse bone marrow. Exp. Hematol. 22, 432–440.
Krysinska, H., Hoogenkamp, M., Ingram, R., Wilson, N., Tagoh, H., Laslo, P., Singh, H., Bonifer, C., 2007. A two-step, PU.1-dependent mechanism for developmentally regulated chromatin remodeling and transcription of the c-fms gene. Mol. Cell Biol. 27, 878–887.
Lam, B.S., Cunningham, C., Adams, G.B., 2011. Pharmacologic modulation of the calcium-sensing receptor enhances hematopoietic stem cell lodgment in the adult bone marrow. Blood 117, 1167–1175. https://doi.org/10.1182/blood-2010-05-286294.
Lane, M.C., Sheets, M.D., 2002. Primitive and definitive blood share a common origin in Xenopus: a comparison of lineage techniques used to construct fate maps. Dev. Biol. 248, 52–67. https://doi.org/10.1006/dbio.2002.0717.
Lichanska, A.M., Browne, C.M., Henkel, G.W., Murphy, K.M., Ostrowski, M.C., McKercher, S.R., Maki, R.A., Hume, D.A., 1999. Differentiation of the mononuclear phagocyte system during mouse embryogenesis: the role of transcription factor PU.1. Blood 94, 127–138.
Lin, H., Lee, E., Hestir, K., Leo, C., Huang, M., Bosch, E., Halenbeck, R., Wu, G., Zhou, A., Behrens, D., Hollenbaugh, D., Linnemann, T., Qin, M., Wong, J., Chu, K., Doberstein, S.K., Williams, L.T., 2008. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. Science 320, 807–811. https://doi.org/10.1126/science.1154370.
Lin, W., Xu, D., Austin, C.D., Caplazi, P., Senger, K., Sun, Y., Jeet, S., Young, J., Delarosa, D., Suto, E., Huang, Z., Zhang, J., Yan, D., Corzo, C., Barck, K., Rajan, S., Looney, C., Gandham, V., Lesch, J., Liang, W.C., Mai, E., Ngu, H., Ratti, N., Chen, Y., Misner, D., Lin, T., Danilenko, D., Katavolos, P., Doudemont, E., Uppal, H., Eastham, J., Mak, J., de Almeida, P.E., Bao, K., Hadadianpour, A., Keir, M., Carano, R.A.D., Diehl, L., Xu, M., Wu, Y., Weimer, R.M., DeVoss, J., Lee, W.P., Balazs, M., Walsh, K., Alatsis, K.R., Martin, F., Zarrin, A.A., 2019. Function of CSF1 and IL34 in macrophage homeostasis, inflammation, and cancer. Front. Immunol. 10, 2019.
Lopez, D., Lin, L., Monaghan, J.R., Cogle, C.R., Bova, F.J., Maden, M., Scott, E.W., 2014. Mapping hematopoiesis in a fully regenerative vertebrate: the axolotl. Blood 124, 1232–1241. https://doi.org/10.1182/blood-2013-09-526970.
Maniatis, G.M., Ingram, V.M., 1971. Erythropoiesis during amphibian metamorphosis. I. Site of maturation of erythrocytes in Rana catesbeiana. J. Cell. Biol. 49, 372–379.
Manning, M.J., Horton, J.D., 1982. RES structure and function of the Amphibia. In: Cohen, N., Sigel, M.M. (Eds.), Phylogeny and Ontogeny. Springer US, Boston, MA, pp. 423–459. https://doi.org/10.1007/978-1-4684-4166-6_11.
Manos, M.M., 1988. Expression and processing of a recombinant human macrophage colony-stimulating factor in mouse cells. Mol. Cell Biol. 8, 5035–5039.
Marr, S., Morales, H., Bottaro, A., Cooper, M., Flajnik, M., Robert, J., 2007. Localization and differential expression of activation-induced cytidine deaminase in the Amphibian Xenopus upon antigen stimulation and during early development. J. Immunol. 179, 6783–6789.
Meseguer, J., Lozano, M.T., Agulleiro, B., 1985. Ultrastructure of the renal granulopoietic tissue of the Rana ridibunda tadpole. J. Submicr. Cytol. 17, 391–401.
Naik, S.H., Metcalf, D., van Nieuwenhuijze, A., Wicks, I., Wu, L., O'Keeffe, M., Shortman, K., 2006. Intrasplenic steady-state dendritic cell precursors that are distinct from monocytes. Nat. Immunol. 7, 663–671.
Nandi, S., Cioce, M., Yeung, Y.-G., Nieves, E., Tesfa, L., Lin, H., Hsu, A.W., Halenbeck, R., Cheng, H.-Y., Gokhan, S., Mehler, M.F., Stanley, E.R., 2013. Receptor-type protein-tyrosine phosphatase ζ is a functional receptor for interleukin-34. J. Biol. Chem. 288, 21972–21986.
Neumann, N.F., Barreda, D.R., Belosevic, M., 2000. Generation and functional analysis of distinct macrophage sub-populations from goldfish (*Carassius auratus L.*) kidney leukocyte cultures. Fish Shellfish Immunol. 10, 1–20.
Nogawa-Kosaka, N., Sugai, T., Nagasawa, K., Tanizaki, Y., Meguro, M., Aizawa, Y., Maekawa, S., Adachi, M., Kuroki, R., Kato, T., 2011. Identification of erythroid progenitors induced by erythropoietic activity in *Xenopus laevis*. J. Exp. Biol. 214, 921–927.
Orkin, S.H., Zon, L.I., 2008. Hematopoiesis: an evolving paradigm for stem cell biology. Cell 132, 631–644.
O'Rourke, D.P., 2007. Amphibians used in research and teaching. ILAR J. Natl. Res. Counc. Inst. Lab. Anim. Resour. 48, 183–187.
Paquin-Proulx, D., Greenspun, B.C., Kitchen, S.M., Saraiva Raposo, R.A., Nixon, D.F., Grayfer, L., 2018. Human interleukin-34-derived macrophages have increased resistance to HIV-1 infection. Cytokine 111, 272–277.
Popovic, M., Yaparla, A., Paquin-Proulx, D., Koubourli, D.V., Webb, R., Firmani, M., Grayfer, L., 2019. Colony-stimulating factor-1- and interleukin-34-derived macrophages differ in their susceptibility to Mycobacterium marinum. J. Leukoc. Biol. 106, 1257–1269.
Potocnik, A.J., Brakebusch, C., Fässler, R., 2000. Fetal and adult hematopoietic stem cells require beta1 integrin function for colonizing fetal liver, spleen, and bone marrow. Immunity 12, 653–663.
Purrello, M., Scalia, M., Corsaro, C., Di Pietro, C., Piro, S., Sichel, G., 2001. Melanosynthesis, differentiation, and apoptosis in Kupffer cells from Rana esculenta. Pigment Cell Res. Spons. Eur. Soc. Pigment Cell Res. Int. Pigment Cell Soc. 14, 126–131.
Ramakrishnan, L., 2013. The zebrafish guide to tuberculosis immunity and treatment. Cold Spring Harbor Symp. Quant. Biol. 78, 179–192.
Ramakrishnan, L., Federspiel, N.A., Falkow, S., 2000. Granuloma-specific expression of Mycobacterium virulence proteins from the glycine-rich PE-PGRS family. Science 288, 1436–1439.
Rettenmier, C.W., Roussel, M.F., 1988. Differential processing of colony-stimulating factor 1 precursors encoded by two human cDNAs. Mol. Cell Biol. 8, 5026–5034.
Robert, J., Ohta, Y., 2009. Comparative and developmental study of the immune system in Xenopus. Dev. Dyn. Off. Publ. Am. Assoc. Anat. 238, 1249–1270.
Robert, J., Abramowitz, L., Gantress, J., Morales, H.D., 2007. Xenopus laevis: a possible vector of Ranavirus infection? J. Wildl. Dis. 43, 645–652.
Robert, J., Grayfer, L., Edholm, E.S., Ward, B., De Jesus Andino, F., 2014. Inflammation-induced reactivation of the ranavirus Frog Virus 3 in asymptomatic Xenopus laevis. PLoS One 9, e112904.
Rollins-Smith, L.A., 2017. Amphibian immunity-stress, disease, and climate change. Dev. Comp. Immunol. 66, 111–119.
Rosa, A., Ballarino, M., Sorrentino, A., Sthandier, O., De Angelis, F.G., Marchioni, M., Masella, B., Guarini, A., Fatica, A., Peschle, C., Bozzoni, I., 2007. The interplay between the master transcription factor PU.1 and miR-424 regulates human monocyte/macrophage differentiation. Proc. Natl. Acad. Sci. U.S.A. 104, 19849–19854.
Samanta, M., Yim, J., De Jesus Andino, F., Paiola, M., Robert, J., 2021. TLR5-Mediated reactivation of quiescent ranavirus FV3 in Xenopus peritoneal macrophages. J. Virol. 95, e00215-e00221.
Sichel, G., 1988. Biosynthesis and function of melanins in hepatic pigmentary system. Pigment Cell Res. Spons. Eur. Soc. Pigment Cell Res. Int. Pigment Cell Soc. 1, 250–258.
Sichel, G., Scalia, M., Corsaro, C., 2002. Amphibia kupffer cells. Microsc. Res. Tech. 57, 477–490.
Smith-Berdan, S., Nguyen, A., Hassanein, D., Zimmer, M., Ugarte, F., Ciriza, J., Li, D., García-Ojeda, M.E., Hinck, L., Forsberg, E.C., 2011. Robo4 cooperates with Cxcr4 to specify hematopoietic stem cell localization to bone marrow niches. Cell Stem Cell 8, 72–83.
Stanley, E.R., Chen, D.M., Lin, H.S., 1978. Induction of macrophage production and proliferation by a purified colony stimulating factor. Nature 274, 168–170.

Tagoh, H., Himes, R., Clarke, D., Leenen, P.J.M., Riggs, A.D., Hume, D., Bonifer, C., 2002. Transcription factor complex formation and chromatin fine structure alterations at the murine *c-fms* (CSF-1 receptor) locus during maturation of myeloid precursor cells. Genes Dev. 16, 1721–1737.

Tanaka, Y., 1976. Architecture of the marrow vasculature in three amphibian species and its significance in hematopoietic development. Am. J. Anat. 145, 485–497.

Taylor, E.W., Fear, A.L., Bohm, A., Kim, S.H., Koths, K., 1994. Structure-function studies on recombinant human macrophage colony-stimulating factor (M-CSF). J. Biol. Chem. 269, 31171–31177.

Wang, Y., Colonna, M., 2014. Interkeukin-34, a cytokine crucial for the differentiation and maintenance of tissue resident macrophages and Langerhans cells. Eur. J. Immunol. 44, 1575–1581.

Wang, T., Hanington, P.C., Belosevic, M., Secombes, C.J., 2008. Two macrophage colony-stimulating factor genes exist in fish that differ in gene organization and are differentially expressed. J. Immunol. Baltim. Md 1950 181, 3310–3322.

WHO, 2016. Global Tuberculosis Report.

Yaparla, A., Wendel, E.S., Grayfer, L., 2016. The unique myelopoiesis strategy of the amphibian *Xenopus laevis*. Dev. Comp. Immunol. 63, 136–143.

Yaparla, A., Popovic, M., Grayfer, L., 2018. Differentiation-dependent antiviral capacities of amphibian (*Xenopus laevis*) macrophages. J. Biol. Chem. 293, 1736–1744.

Yaparla, A., Docter-Loeb, H., Melnyk, M.L.S., Batheja, A., Grayfer, L., 2019. The amphibian (*Xenopus laevis*) colony-stimulating factor-1 and interleukin-34-derived macrophages possess disparate pathogen recognition capacities. Dev. Comp. Immunol. 98, 89–97.

Yaparla, A., Reeves, P., Grayfer, L., 2020. Myelopoiesis of the amphibian *Xenopus laevis* is segregated to the bone marrow, away from their hematopoietic peripheral liver. Front. Immunol. 10.

Zettergren, L.D., 1982. Ontogeny and distribution of cells in B lineage in the American leopard frog, Rana pipiens. Dev. Comp. Immunol. 6, 311–320.