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Amphibians as a model to study the role of immune cell heterogeneity in host and mycobacterial interactions

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ABSTRACT

Mycobacterial infections represent major concerns for aquatic and terrestrial vertebrates including humans. Although our current knowledge is mostly restricted to *Mycobacterium tuberculosis* and mammalian host interactions, increasing evidence suggests common features in endo- and ectothermic animals infected with non-tuberculous mycobacteria (NTMs) like those described for *M. tuberculosis*. Importantly, most of the pathogenic and non-pathogenic NTMs detected in amphibians from wild, farmed, and research facilities represent, in addition to the potential economic loss, a rising concern for human health. Upon mycobacterial infection in mammals, the protective immune responses involving the innate and adaptive immune systems are highly complex and therefore not fully understood. This complexity results from the versatility and resilience of mycobacteria to hostile conditions as well as from the immune cell heterogeneity arising from the distinct developmental origins according with the concept of layered immunity. Similar to the differing responses of neonates versus adults during tuberculosis development, the pathogenesis and inflammatory responses are stage-specific in *Xenopus laevis* during infection by the NTM *M. marinum*. That is, both in human fetal and neonatal development and in tadpole development, responses are characterized by hypo-responsiveness and a lower capacity to contain mycobacterial infections. Similar to a mammalian fetus and neonates, T cells and myeloid cells in *Xenopus* tadpoles and axolotls are different from the adult immune cells. Fetal and amphibian larval T cells, which are characterized by a lower T cell receptor (TCR) repertoire diversity, are biased toward regulatory function, and they have distinct progenitor origins from those of the adult immune cells. Some early developing T cells and likely macrophage subpopulations are conserved in adult anurans and mammals, and therefore, they likely play an important role in the host-pathogen interactions from early stages of development to adulthood. Thus, we propose the use of developing amphibians, which have the advantage of being free-living early in their development, as an alternative and complementary model to study the role of immune cell heterogeneity in host-mycobacteria interactions.

1. Introduction

The aim of this review is to present the advantages of amphibians as model organisms for investigating the role of immune cell heterogeneity in host and mycobacteria interactions. First, we provide a brief update on mycobacterial diversity and biology with special emphasis on the features that make *Mycobacterium tuberculosis* and non-tuberculous mycobacteria (NTM) serious concerns for human, wildlife, and animal productions including fish and amphibian farming. Subsequently, we provide an overview of what is known about the interaction between NTMs and developing amphibian hosts and discuss the use of amphibians as models to investigate the role of ontogenesis related-immune cell heterogeneity with a particular focus on T cells (*i.e.,* layer immunity) in mycobacteria-host interaction.

2. Diversity of mycobacteria and mycobacterial diseases

Mycobacteria are a diverse group of aerobic, non-motile, acid fast bacilli comprising nearly 200 species that are mainly non-pathogenic environmental saprophytes (Parte, 2014; Tortoli et al., 2019; Turenne, 2019). A fraction of the mycobacterial species has evolved as opportunistic or obligate pathogens, which represent serious concerns for human health, wildlife conservation, and economy (aquaculture and research involving animal experimentation). These mycobacterial

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species can be classified in three groups based on their pathogenicity and their dependence on a host for replication (reviewed in Gagneux, 2018; Johansen et al., 2020; Pereira et al., 2020). The best described are the *Mycobacterium tuberculosis* complex, which encompass numerous closely related species that are the causative agents of tuberculosis in mammals (*e.g., M. tuberculosis* (*Mtb*) in humans and *M. bovis* in cattle). *Mtb* are considered as one of the most adapted pathogens to their hosts due to a long co-evolutionary history (Gagneux, 2018). They are strictly pathogenic, depending on the host to survive and propagate (Gagneux, 2018). Before the outbreak of COVID-19, *Mtb* was the leading cause of human death due to a single pathogenic agent (WHO, 2021). The second group is represented by *M. leprae*, and *M. lepromatosis* which are obligate pathogens and cause leprosy in humans (Johansen et al., 2020; Turenne, 2019). The third group is called non-tuberculous mycobacteria (NTM). NTMs are ubiquitously found in the environment (water, air, and soil), and can cause opportunistic tuberculosis-like diseases in a wide range of hosts from humans to birds, reptiles, amphibians and fish (reviewed in Johansen et al., 2020; Pereira et al., 2020; Turenne, 2019). Most investigated NTMs are represented by complexes of *M. marinum-ulcerans*, *M. avium*, and *M. abscessus-chelonae* (Reviewed in Hodgkinson et al., 2019; Johansen et al., 2020; Turenne, 2019).

The mycobacterial structure is characterized by a complex cell envelope consisting of a mycolyl-arabinogalactan-peptidoglycan cell wall with an outer so-called mycomembrane that forms the capsule surrounding the periplasm and plasma membrane (Reviewed in Dulberger et al., 2020). The mycobacterial cell envelope is an impermeable, robust and dynamic wall of protection, which drastically impacts host immune responses and pathogenicity as well as the pathogen sensitivity to antibiotics (Dulberger et al., 2020; Johansen et al., 2020). Besides a natural tolerance to numerous antibiotics (*e.g.,* Quinolones and β-lactams), the hydrophobicity of their envelope favors surface attachment, aerosolization, and it gives the capacity of mycobacteria to aggregate as clump or serpentine cords (Reviewed in Jarlier and Nikaido, 1994; Johansen et al., 2020; Pereira et al., 2020). Aggregate formation contributes to the virulence by impairing phagocytosis, promoting the death of phagocytes, and favoring the progression toward active disease (Johansen et al., 2020; Julián et al., 2010; Kolloli et al., 2021). Furthermore, various environmental stresses (*e.g.,* carbon/nutrient availability, oxidative stress, metal ion availability, pH changes) stimulate mycobacteria to form biofilms, which affects pathogenicity and tolerance to sanitizers and antibiotics (Belardinelli et al., 2021; Chakraborty et al., 2021), reviewed in (Esteban and García-Coca, 2018). Bacteria from the *Mtb* complex are transmitted among individuals by aerosol droplets following coughing. Consequently, *Mtb* primarily infect the lungs from which they can disseminate to any tissues. In contrast, NTM infections are generally not transmitted from host to host but result from environmental exposure (Johansen et al., 2020; Pereira et al., 2020; Turenne, 2019). NTM infections can occur by inhalation, swallowing, and wound contact (Johansen et al., 2020; Pereira et al., 2020). Like *Mtb*, infecting NTMs are recognized *via* complex interactions between Microbe Associated Molecular Patterns (MAMPs) and Pathogen Recognition Receptors (PRRs) expressed by macrophages and neutrophils (Chai et al., 2020; Dubé et al., 2021; Johansen et al., 2020; Lovewell et al., 2021). Upon phagocytosis, the bacilli can persist and proliferate in these innate immune cells (Chai et al., 2020; Dubé et al., 2021; Johansen et al., 2020; Lovewell et al., 2021). Activation of PRRs initiates the production of pro-inflammatory cytokines (IL-1β, type I IFNs and TNF-alpha) that are protective but also triggers histopathology such as granulomas (Bernut et al., 2016, reviewed in Chai et al., 2019; Johansen et al., 2020). Although mycobacteria are generally cleared by the host immune system, they may be resistant to stressful conditions triggered by the host antimicrobial defenses (*e.g.,* hypoxia and oxidative damage) or bactericidal drugs by forming biofilms, manipulating cellular innate immune defenses, and entering into dormancy (Chakraborty et al., 2021 reviewed in Chai et al., 2020; Chao and Rubin, 2010; Pereira et al., 2020). Dormancy is an adaptation during which bacteria do not replicate (Chao and Rubin, 2010; Pereira et al., 2020). Two types of dormant states arising from natural population heterogeneity have been described for bacteria including *Mtb*: (1) a persistent state; and (2) a viable but nonculturable state (reviewed in Ayrapetyan et al., 2015; Chao and Rubin, 2010; Chung et al., 2022). Distinct from "persister", the viable but nonculturable bacteria are unable to "resuscitate" and form colonies on nutrient-rich solids used as a standard assay to detect *Mtb* (Ayrapetyan et al., 2015; Chengalroyen et al., 2016; Saito et al., 2021). Similarly, NTM such as *M. marinum, M. avium, M. smegmatis* and *M. abscessus* persist in the host under stressful conditions by stopping their replication until favorable conditions allow reactivation from their dormant state (Archuleta et al., 2005; Commandeur et al., 2020; Dick et al., 1998; Yam et al., 2020). Under favorable conditions NTMs reactivate from their dormant state indicative of a persistent state (Archuleta et al., 2005; Commandeur et al., 2020; Dick et al., 1998). Additionally, *M. smegmatis* like *Mtb* can develop a nonculturable state suggesting that the two dormant states are conserved in NTMs as well as in *Mtb* (Shleeva et al., 2004). Mycobacterial infections are typically contained by the host in characteristic inflammatory structures called granulomas, which are formed by the aggregation of fibrous material and immune cells (neutrophils, macrophages and lymphocytes) eventually creating a central necrotic core (Johansen et al., 2020). Dormant mycobacteria are capable of reactivation or resuscitation after a prolonged time when the host immune system controls are lifted (*i.e.,* immunosuppression triggered by HIV infection or anti-TNF therapy) to resume replication and cause active disease (Myllymäki et al., 2018; reviewed in Boom et al., 2021; Chao and Rubin, 2010). Dormancy is often associated with latency, which is defined as the state in which chronically infected individuals are asymptomatic with a persistent immune response (Reviewed in Behr et al., 2021; Chao and Rubin, 2010). The dormant state has a major role in the wide success of mycobacteria as pathogens. In the case of *Mtb*, it is estimated that one quarter of the world's population is asymptomatically infected, creating a huge potential pathogen reservoir (WHO, 2021). Importantly, the intrinsic capacity of mycobacteria to tolerate multiple bactericidal drugs (*e.g.,* dormancy, biofilm formation, wall thickening, efflux pumps and drug metabolism) favors the emergence of multiple drug resistance mutations (Dutta et al., 2019; Liu et al., 2020; reviewed in Bakkeren et al., 2020; Goossens et al., 2020; Saxena et al., 2021). Interestingly, during the dormant state, *Mtb* continues to accumulate mutations with the same rate as during the active replication state because of the host-directed oxidative damages (Ford et al., 2011). Such phenomena are likely to play, and have played, a key role in the adaptation of *Mtb* and potentially other mycobacteria to environmental pressure (metabolic constraint and antibiotic resistance). In addition to clonal variation, horizontal gene transfer within mycobacteria or within the microbial community play a key role in the evolution of environmental mycobacteria (Pereira et al., 2020).

3. Mycobacteriosis in amphibians

Over recent years, the human incidence of NTM infections has increased over *Mtb* in several developed countries and represents a growing health concern for immunocompromised patients (Chai et al., 2022; Johansen et al., 2020). In animals, data on the incidence of NTMs are limited to sporadic case studies (Biet and Boschiroli, 2014; Martinho and Heatley, 2012; Pereira et al., 2020). However, NTM infections have been reported in multiple economically important farmed animals (cattle, sheep, pigs, poultry, and fish), research facilities (zebrafish and frogs) and wild animals (amphibians, birds) (reviewed in Biet and Boschiroli, 2014; Pereira et al., 2020).

Cases of mycobacterial infection in amphibians have been reviewed elsewhere (Martinho and Heatley, 2012). Most reports are limited to captive frogs such as *Xenopus* colonies (*X. laevis* and *X. tropicalis*) and bullfrogs (*Rana catesbeiana*). These species are widely used and are raised in large colonies for biomedical research or meat production, respectively (FAO, 2022). Based on genome sequences, it was estimated that Pipids (*Xenopus*) and Ranids diverge over 200 million years ago while *X. laevis* and *X. tropicalis* diverged about 48 million years ago (Hammond et al., 2017; Session et al., 2016). *Mycobacterium* spp. have been detected in frog skin and tadpole intestine in laboratory and wild conditions (Costa et al., 2016; Huang et al., 2021; Kohl and Yahn, 2016). Amphibian mycobacterial infections have been reported in zoos or in the wild. *M. marinum-ulcerans* complex is causing the most devastating disease and is responsible for important economic losses (Martinho and Heatley, 2012). *M. ulcerans* Ecovar Liflandii (*i.e.*, an ecotype of *M. ulcerans* the causative agent for Buruli ulcer in humans) is responsible for devastating mortality outbreaks in research facilities housing *X. laevis* and *X. tropicalis* colonies in United States and Europe (Tobias et al., 2013). Although *M. ulcerans* Ecovar Liflandii is not known as a human pathogen, it has been described as responsible of disease outbreak in farmed fish (Luo et al., 2022; Zhang et al., 2018). Multiple NTMs have been reported to infect amphibians, including species from the complexes of *M. chelonae-abscessus* and *M. avium* (Barrows et al., 2017; Martinho and Heatley, 2012; Slater et al., 2021). Importantly, among mycobacteria causing disease in amphibians (*e.g., M. bovis, M. szulgai, M. marinum, M. xenopi* and *M. fortuitum*), only *M. ulcerans* Ecovar Liflandii has not been reported to date as an opportunistic pathogens in humans and mammalian livestock (Gcebe et al., 2018; Haridy et al., 2014; Ikuta et al., 2018; Martinho and Heatley, 2012; Pereira et al., 2020). *M. ulcerans* causing Buruli ulcer in humans has also been detected in wild anurans. Although, *M. ulcerans* hasn't been reported as a pathogen for amphibians or fish, it represents a potential reservoir for human disease (Garchitorena et al., 2014; Willson et al., 2013).

NTMs are ubiquitous in the environment including aquatic animal research facilities and households (Mason et al., 2016; Pereira et al., 2020). Drinking water systems are an important source and reservoir for NTMs where these pathogens can persist in biofilms (plumbing systems, sinks, tanks, and hoses). Food and amoebae represent other sources of NTMs (Chang et al., 2019; Delafont et al., 2014; Mason et al., 2016; reviewed in Honda et al., 2018; Pereira et al., 2020). Thus, NTMs are unlikely to be completely eradicated from research facilities or aquaculture farms, and strategies to mitigate pathogen outbreaks should be considered (*e.g.,* environmental sampling, sentinel animals, NTM adapted sanitizers and removal of the elder individuals; Mason et al., 2016).

An additional challenge is that infections by NTMs, like *Mtb*, frequently persist at a sub-clinical or latent state (*i.e.,* chronic infection; Chai et al., 2012; Martinho and Heatley, 2012). Mortality or disease outbreaks typically occur under stressful conditions (*e.g.,* husbandry facility dysfunction, co-infection from another pathogen and high density), which impairs the host immune system and triggers acute infections resulting in high death rates (Chai et al., 2012; Martinho and Heatley, 2012; Ramsay et al., 2009). The first signs of an active mycobacterial infection include loss of weight, anorexia, lethargy, edema, and skin ulcers. A typical feature associated with mycobacterial infection is the formation of granulomas in organs such as liver, kidney, and lungs, as well as skin, depending of the route of infection and the spreading of the infection (Chai et al., 2012; Haridy et al., 2014; Ikuta et al., 2018; Martinho and Heatley, 2012).

3.1. Host-mycobacterial interactions and layered immunity

Upon infection of mammalian species, mycobacteria are rapidly recognized and phagocyted by macrophages and neutrophils, which trigger innate immune signals that recruit various waves of immune cells to eradicate the pathogenic agent. First responders are innate immune cells such as monocyte-derived macrophages, neutrophils, dendritic cells, natural killer cells and innate-like T cells (reviewed in Chai et al., 2019). Subsequently, upon their activation by antigen-presenting cells in secondary lymphoid organs, adaptive lymphocytes (B cells, conventional CD8 and CD4 T cells) differentiate and expand into effectors before their recruitment to the infection site (reviewed in Ankley et al., 2020; Chai et al., 2019). The role of the different immune effector cell populations in the outcome of the disease (clearance vs. persistence) is very complex and still poorly understood owing to virulence factors used by mycobacteria to evade or impair immune defenses (reviewed in Ankley et al., 2020; Chai et al., 2020; Ernst, 2018).

Mtb evades conventional T cell responses by acting at various levels (Ankley et al., 2020; Chai et al., 2020; Chen and Flies, 2013; Ernst, 2018). In mammals, *Mtb* targets the MHC-II antigen presentation by blocking IFNɣ mediated MHC-II upregulation, by interfering with mycobacteria-derived antigen-MHC-II assembly, and by impairing autophagy and phagosome maturation (Ankley et al., 2020; Chai et al., 2020; Ernst, 2018). Furthermore, *Mtb* can still impair T cell function at the peak of the adaptive response by promoting regulatory T cells (Treg) differentiation and T cell exhaustion (Gern et al., 2021; Jayaraman et al., 2016; Shafiani et al., 2013: reviewed in Ankley et al., 2020; Ernst, 2018; Verma et al., 2021). As a result, T cell priming toward *Mtb* is slow in comparison to other pathogens, and primed T cells are not very efficient in recognizing *Mtb* infected macrophages (Chackerian et al., 2002; Patankar et al., 2020). Additionally, although granulomas are important to contain mycobacterial infection, peripheral confinement of T cells likely limits their efficacy because T cells cannot be directly activated by infected cells (Ankley et al., 2020; Ernst, 2018).

Unconventional T cells, also referred as pre-set or innate-like T cells (iT cells), also play a critical role in the host immune response against *Mtb* (reviewed in Huang, 2016; Ruibal et al., 2021). In mammals, iT cells represent a distinct and heterogenous group of T cells including CD1-restricted invariant Natural killer T cells (iNKT cells) and MR1-restricted mucosa associated invariant T cells (MAIT cells) (reviewed in Mayassi et al., 2021). Unlike conventional T cells, iT cells exhibit low TCR diversity and interact with conserved molecular pattern ligands presented by non-polymorphic MHC-I like molecules. iT cells develop early during ontogenesis and can rapidly respond upon activation. iT cells are important for tissue homeostasis and protection and thus, complement the action of conventional T cell responses (reviewed in Constantinides and Belkaid, 2021; Mayassi et al., 2021). Interestingly, MAIT cell stimulation can either negatively or positively contribute to conventional T cell responses against pathogens depending on the timing of the stimulation (*i.e.*, prior to the infection or under chronic infection; Sakai et al., 2021). The full cellular mechanisms behind these dichotomous actions remains largely unknown but may be related to MAIT cells function in tissue homeostasis and repair (Sakai et al., 2021). As reviewed by Angelidou et al. (2020), the importance of ontogenesis on T cell responses and protective immunity during mycobacterial infection is underscored by the different and inconsistent T cell response obtained by vaccination with the Bacilli Calmette-Guérin (BCG) in newborns, infants, children, and adults (Burl et al., 2010; Lutwama et al., 2014; Ritz et al., 2016; Whittaker et al., 2018). Depending on the developmental stage and presumably the genetic background of the human population as well as the BCG strain, different memory T cell frequencies and BCG specific T cell cytokine production were detected. BCG represents the only approved vaccine that provides protection in children (WHO, 2021), which is crucial because children are more susceptible to tuberculosis than adults. Nevertheless, the cellular mechanisms responsible for children's susceptibility to *Mtb* remain largely unknown (reviewed in Basu Roy et al., 2019; Vanden Driessche et al., 2013).

The establishment of the mammalian adult immune system is thought to result from the timed succession of non-redundant phases in a particular order, which are marked by waves of distinct hematopoietic progenitors differentiating into distinct mature immune cell populations (reviewed in Hornef and Torow, 2020; Park et al., 2020). This defines the concept of layered immunity in which the establishment of immune homeostasis, immune competence, and host-microbial interaction depend on an early developmental window (Hornef and Torow, 2020; Park et al., 2020). For example, different tissues resident immune cells (macrophages and iT cells) differentiate during human fetal development, which lay the foundation for postnatal immunity (reviewed in Feyaerts et al., 2022; Miah et al., 2021). In mice and in humans, increasing evidence suggests that the neonatal/fetal immune cells are phenotypically and functionally distinct from those of adults (Carey et al., 2016; Mold et al., 2010; Smith et al., 2018; reviewed in Bennett and Bennett, 2020; Davenport et al., 2020; Miah et al., 2021; Rudd, 2020). Although pre/neonatal immune cells can respond to infection, their effector functions must be tightly regulated to prevent excessive impairment of vital functions and avoid tissue damage (reviewed in Oschwald et al., 2020; Zhang et al., 2017). In contrast to macrophages and DCs, whose phenotypes are highly influenced by the tissue microenvironment (*e.g.,* cytokines, nutrients, microbiota), neonatal naive T cell phenotypes have been suggested to be intrinsic (Bain and MacDonald, 2022; Bennett and Bennett, 2020; Papaioannou et al., 2021; Smith et al., 2018; Wang et al., 2016). However, a recent transcriptomic analysis in humans is challenging this view by indicating that naive human T cells follow a progressive maturation during late fetal development (Bunis et al., 2021). While layered development of naive T cells needs to be further investigated, populations of unconventional T cells such as tissue resident γδ T cells and MAIT cells are generated and functional at early developmental stages (Constantinides et al., 2019; Di Marco Barros et al., 2016; Tieppo et al., 2019; reviewed in (Constantinides and Belkaid, 2021; Mayassi et al., 2021; Ribot et al., 2021). Thus, it remains conceivable that conventional and unconventional neonatal T cells persisting until adulthood might conserve distinctive phenotypes and function, which could complement adult-generated T cells in immune competence, tissue homeostasis and pathogenesis (Constantinides and Belkaid, 2021; Smith et al., 2018; reviewed in Davenport et al., 2020; Rudd, 2020).

4. Amphibians and layered immune systems: A study case of *M. marinum* infection in *X. laevis*

After an overview about mycobacteria and host immune interactions in mammals, we now turn to amphibians, especially *X. laevis*, as alternative models to study the layered development of immune defenses against mycobacteria with special emphasis on T cells.

Amphibians represent a heterogenous group comprising three orders: the Gymnophiona (caecilians); Urodela (salamanders and newts); and Anura (frogs and toads). Gymnophiona are limbless and vermiform. Urodela have elongated bodies, distinct heads, necks, well-developed limbs, and a tail which remains through life. Anura have robust bodies; they are tailless, neckless with highly developed hindlimbs specialized in propulsion (jump and swimming). Amphibians evolved around 350 million years ago and represent early tetrapods as well as the transition group from water to land. Although some species keep the larval form for their entire life (neoteny), skip the larval stage (direct development), or are viviparous, many amphibians transit from the free aquatic larval stage to the adult form (San Mauro et al., 2014). This transition state is referred to metamorphosis. The extent of the morphological changes vary considerably among the orders of amphibians: Anura undergo the most dramatic changes, while Urodela and Gymnophiona retain numerous larval features (reviewed in Alibardi, 2019; Wake, 2006). Similar to the transition of neonatal and infant stages to adulthood in mammals, metamorphosis marks the endocrine-controlled transition from larval to adult frog immune systems (reviewed in Robert and Ohta, 2009; Rollins-Smith, 1998). Like fetal and human neonates, the larval immune system in *Xenopus* is distinct from the adult counterpart. Although *X. laevis* tadpoles show delayed and hypo-immune responsiveness in comparison to one-year-old adults, they are immunocompetent. Tadpoles can develop active immune responses including effector functions during viral infections, immune tolerance during allograft/tumor rejection, and tissue regeneration (Aztekin et al., 2020, 2019; Edholm et al., 2019; Kalia et al., 2022; Rhoo et al., 2019; reviewed in Banach and Robert, 2019; Robert et al., 2017; Robert and Ohta, 2009; Rollins-Smith, 1998). In larval wood frogs, virus loads, but not prevalence of infection, appear to be dependent on MHC- Class II alleles (Savage et al., 2019). Similar to newborns with group B *Streptococcus* and respiratory syncytial virus infections, anuran tadpoles, and especially metamorphic-stage frogs, are more susceptible than adult frogs to pathogens such as ranaviruses (Kalia et al., 2022), reviewed in (Chen and Robert, 2011; Kollmann et al., 2020).

Urodeles retain numerous larval phenotypic features after metamorphosis. Therefore, the immune system doesn't show such drastic changes as in the Anura. Similar to tadpoles, the urodele immune system shows a weak and delayed immune response to pathogens and graft rejection (reviewed in Chen and Robert, 2011; Rollins-Smith, 1998). As a consequence, like anuran tadpoles, salamanders (*e.g.,* Ambystoma) show high susceptibility to ranavirus infection in comparison to adult anurans (Chen and Robert, 2011). In the following sections, we describe (1) our current understanding at the cellular and molecular levels of the developing larval immune function, phenotype, and regulation in the context of infection and (2) evidence showing that amphibians represent a model of interest to study the role of layered immunity in the host-mycobacteria interaction because larval immune cells persist in the adult immune system.

a. Anuran amphibian larval T cells and macrophages are distinct from adult-derived cells and are biased toward tolerance and tissue homeostasis/repair
    i. T cells

As previously reviewed by Benedict et al. (2000), Rudd (2020), Zemlin et al. (2002), mammalian fetal T cells are defined by different TCR and B cell receptor (BCR) repertoires accounting for different gene usages and a constrained complementarity-determining region 3 (CDR3) in comparison to their adult counterparts (Carey et al., 2016; Rechavi et al., 2015). After birth for rodents and during gestation in human, Terminal deoxynucleotidyl transferase (TdT) expression increases greatly in the thymus of rat, mice and likely human, together with the increase of TCR and BCR N-region diversity (Bogue et al., 1992; Carey et al., 2016; Carisson and Holmberg, 1990; Gregoire et al., 1979; Rechavi et al., 2015; Souto-Carneiro et al., 2005). In *Xenopus,* TdT mRNA was detected at a low level in the thymus of tadpoles but is substantially increased during metamorphosis and in adults together with N-region diversity in the B cell repertoire (Lee and Hsu, 1994; Schwager et al., 1991). In two-week-old *Xenopus* tadpoles (stage 50–51), the TCR repertoire of CD8^{neg} and CD8^{dim} T cells is dominated by six invariant TCR alpha chains (Edholm et al., 2013). These data indicate that *Xenopus* TCR and BCR repertoires are constrained in terms of CDR3 length and segment usage before metamorphosis similar to mammalian fetal T cells. In the Mexican axolotl (a urodele), TdT transcripts are detected in the thymus from the larval to adult stages (Golub et al., 2004). Furthermore, the BCR and TCR repertoire diversity is apparently low without major changes occurring during axolotl ontogenesis. The TCR N-region diversity does not drastically change during development, and the TCRβ repertoire is highly shared among individuals and between larval and adult stages, *i.e.,* public TCRβ repertoire (Kerfourn et al., 1996; Schwager et al., 1991). Interestingly, *tdt* deficiency in mice reduces the TCR repertoire diversity and peptide specificity, while it favors polyreactive TCR, promiscuous peptide recognition, and public T cell repertoire, which is reminiscent of fetal and neonatal B and T cells (Benedict et al., 2000; Davenport et al., 2020; Gavin and Bevan, 1995). This convergence in low TCR diversity and cross-reactivity among mammalian fetal T cells, anuran tadpole T cells, and urodelan T cells appears to be related to the constraints of (1) generating a TCR repertoire with a small number of T cells (Giorgetti et al., 2021) and (2) limiting autoreactivity against self-antigens (Reviewed by Rudd, 2020) and (3) limiting a strong and harmful T cell inflammatory responses (*i.e.,* cytokine production and clonal expansion) against non-self-antigens (*i.*

e., tolerance; Carey et al., 2016; Schreurs et al., 2021; Stutz et al., 2021).

The difference in TCR repertoire between *X. laevis* tadpole and adult T cells is also characterized by a difference in TCR restriction and reactivity. Unlike the mammalian fetus, *X. laevis* tadpoles are deprived of MHC-Class Ia restricted T cells because MHC-Class Ia surface protein expression appears at the onset of metamorphosis; Flajnik et al., 1986). As previously speculated (Reviewed in Flajnik et al., 1987), the absence of MHC-Class I at the protein level might represent an adaptation to the small number of T cells present in *Xenopus* tadpoles. The development of both MHC-Class II and MHC-Class I restricted T cells would require a higher number of cells. In *X. laevis*, metamorphosis is marked by a drastic loss of thymocytes and splenic T cells, which are replenished a few weeks after metamorphosis in young froglets (Du Pasquier and Weiss, 1973; Robert and Ohta, 2009; Rollins-Smith, 1998). Like mammalian neonatal T cells that must be educated to distinguish between beneficial and pathogenic microbiota, the metamorphic froglet T cells must learn to tolerate newly synthetized adult proteins as well as new food-derived antigens since metamorphosis is also marked by a change in the diet and microbiota (Reviewed in Colombo et al., 2015; Robert and Ohta, 2009). The drastic loss of lymphocytes in the thymus and spleen during metamorphosis is postulated to be a mechanism of central and peripheral tolerance/selection. The latter would eliminate larval immune cells that would be autoreactive to the newly generated adult-derived antigens. In urodeles, the change associated with metamorphosis remains largely uncharacterized. In urodele larvae, although MHC Class Ia is expressed at the transcript level as in *X. laevis* tadpoles, the protein expression is unknown due to the lack of reagents (Salter-Cid et al., 1998; Tournefier et al., 1998). MHC Class II shows developmental stage specific cellular distribution in *Xenopus* as in the axolotl (Rollins-Smith and Blair, 1990; Völk et al., 1998). Indeed, most T cells and thymocytes express MHC-Class II only after metamorphosis in *X. laevis* and axolotl (Rollins-Smith and Blair, 1990; Völk et al., 1998). Constitutive expression of MHC Class II by T cells and thymocytes has also been detected in the horse (Lunn et al., 1993). In humans and rats, MHC Class II is expressed mainly on activated peripheral T cells, while mice lack MHC Class II expression on thymocytes and T cells (Reviewed by Lee et al., 2009). Although, the role of MHC Class II on thymocytes and T cells remains to be characterized in *X. laevis* (Reviewed in human by Lee et al., 2009), the absence of surface MHC Class II expression on tadpole thymocytes and T cells, as well as process of central/peripheral tolerance during metamorphosis, impies that tadpole and adult T cells have a distinct TCR specificity.

Human infant and *X. laevis* tadpole T cell hypo-responsiveness likely limits the capacity to control mycobacterial infection. Infants show a general diminished T cell-mediated response including cytokine secretions (*e.g.,* IL-1β, TNFα) and delayed T cell responses to BCG (Reviewed in Basu Roy et al., 2019; Vanden Driessche et al., 2013). Although *X. laevis* adult frogs produce a strong pro-inflammatory CD8 T cells response against *M. marinum,* including the up-regulation of IL-1β and TNFα gene transcripts and the formation large granulomas, tadpole immune responses are characterized by a prominent iT cell response, a minimal pro-inflammatory gene expression change, and enhanced expression of anti-inflammatory genes such as IL-10 (Rhoo et al., 2019). Children under five years of age also exhibit reduced cavity formation (*i. e.,* the ultimate granuloma state), higher risk of developing the disseminated form of tuberculosis (*e.g.,* meningitis and miliary tuberculosis), and a higher risk of developing active disease in comparison to adults (Basu Roy et al., 2019; Vanden Driessche et al., 2013). Similarly, tadpole infection by *M. marinum* is characterized by the formation of fewer microgranulomas surrounded by rare T cells and persistence of higher pathogen loads in comparison to adult frogs (Rhoo et al., 2019). Importantly, tadpoles and adult frogs do not show a statistical difference in their survival rate during mycobacterial challenges (Rhoo et al., 2019). This is unlike children under five year of age who are more likely to die from tuberculosis than adults (Basu Roy et al., 2019; Vanden Driessche et al., 2013). Altogether, these data suggest some similarity between the infant and tadpole immune systems including T cell hypo-responsiveness to mycobacterial infection and increased bacterial dissemination in comparison to the adult counterpart.

Functionally, neonatal T cells are intrinsically biased toward tolerance compared to their adult counterparts (Davenport et al., 2020; Rudd, 2020). Interestingly, mammalian neonatal Tregs and likely urodele and anuran larval T cells show important regeneration capacity that is lost in older infants and in adult anurans after metamorphosis (Aztekin et al., 2020; Fukazawa et al., 2009; Leigh et al., 2018; Li et al., 2019). Furthermore, adult allograft rejection can be delayed by injection of thymocytes derived from metamorphic isogenic frogs supporting the immunoregulatory function of pre-adult T cells (Du pasquier and Bernard, 1980). The difference of metamorphosis between Urodela and Anura coincides with different capacities for tissue regeneration. Urodeles retain an extended capacity for tissue regeneration throughout life, whereas this capacity is highly reduced after metamorphosis in anurans (Godwin and Rosenthal, 2014). In newts, treatment with the T cell-inhibitor Cyclosporin A inhibited tissue regeneration (Fahmy and Sicard, 2002) and, in axolotls, thymectomy in juveniles and adults boosted antibody responses (Charlemagne, 1979). These data suggested that putative urodele Tregs may contribute to the retention of an extended regeneration capacity after metamorphosis. Thus, similar to fetal/neonatal T cells, tadpole/metamorphic frog and axolotl T cells are likely distinct from their adult counterparts and are biased toward an immunoregulatory function and anti-inflammatory activity. Such biases likely limit the capacity to restrain mycobacterial infection in *X. laevis* tadpoles and neonates/infants.

ii. Innate-like T cells have a major role in *X. laevis* larval immune defenses

MHC Class Ib restricted T cell similar to mammalian MAIT and iNKT cells have been functionally described outside mammals only in *X. laevis* to date (Edholm et al., 2013, 2018). *Xenopus* iT cells share some ontogenetic and phenotypic features with mammalian MAIT cells and iNKT cells as previously reviewed (Banach and Robert, 2019; Hyoe and Robert, 2019). Inferring the phylogeny of MHC Class I related genes in jawed vertebrates is complex because, although they are present in all investigated species, their rapid evolution rate often hampers the establishment of a clear orthology (Almeida et al., 2020, 2021; Boudinot et al., 2016; Goyos et al., 2011). Therefore, the evolutionary relationship between *X. laevis* and mammalian iT cells remains unknown.

The limited capacity to restrain mycobacterial infections of tadpoles and neonates might be related to the prominence of iT cells (Mayassi et al., 2021; Sakai et al., 2021). Tadpole survival against *M. marinum* is critically dependent on the MHC1b-UBA4 interacting with iT cells expressing the invariant and germ-line encoded Vα45-Jα1.14 (iVα45; Edholm et al., 2018). Using short hairpin RNAs (shRNA) targeting the CDR3, the iVα45 was shown to be important for these cells for liver homeostasis, which raises the possibility that iVα45 deficiency compromises tadpole survival by impacting liver repair during infection (Edholm et al., 2018; Rhoo et al., 2019). Consistent with the prominent role of MHC Class Ib-restricted iT cells in tadpole's resistance, tissue homeostasis and repair, tadpole immune resistance might rely on the tolerance of higher mycobacterial pathogen loads and hypo-reactivity (Edholm et al., 2018; Rhoo et al., 2019).

iii. Myeloid cells

Macrophages, and to a lesser extent neutrophils, are central for tissue repair, viral and mycobacterial infection in *Xenopus* and axolotls as in mammals (Aztekin et al., 2020; Fukazawa et al., 2009; Leigh et al., 2018; Popovic et al., 2019). Notably, macrophage polarization by two evolutionarily unrelated ligands of the Colony stimulating factor 1 receptor (Csf1r), Csf1 and Interleukin-34 (IL-34), drastically impact anti-viral and anti-mycobacterial immune responses in adult *X. laevis* (Grayfer and

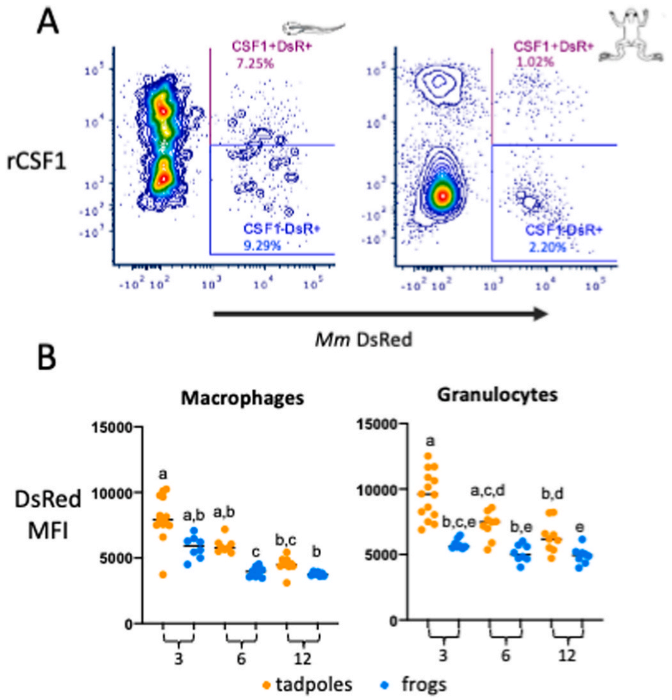

Fig. 1. Kinetics of *M. marinum* pathogen load in the peritoneal myeloid cells in adult and tadpole *Xenopus* by flow cytometry. For this study, 3 × 10^5 CFU and 1 × 10^6 CFU of DsRed + *M. marinum* were inoculated in the peritoneum of *X. laevis* tadpoles and adult frogs, respectively. (A) Representative flow cytograms of adults and tadpoles showing DsRed + *M. marinum* signal in peritoneal CSF1R + macrophages and CSF1R-granulocytes at 3 dpi. (B) DsRed Mean Fluorescence Intensity (MFI) in CSF1R + macrophages and CSF1R-granulocytes from tadpoles and adults at 3, 6 and 12 day post infection as represented by the orange and blue dots respectively. Sampling of peritoneal leucocytes was repeated in the same individual at each time points. n = 6–10 from two independent experiments. a,e indicate statistical significance (Kruskal-Wallis test, $p < 0.05$). rCSF1, recombinant CSF1

Robert, 2015; Popovic et al., 2019). Using the transgenic *mpeg*:GFP *X. laevis* line strain and DsRed + *M. marinum,* the recruitment of GFP + macrophages and the formation of granuloma structure have been monitored in tadpoles (Hyoe and Robert, 2019). Developmental origin and function (*e.g.*, tissue homeostasis) also determines the role of myeloid cells in mycobacterial pathogenicity. For example, *Mtb* evades immune surveillance by taking advantage of the more permissive lung alveolar macrophages in comparison to interstitial macrophages and neutrophils (Huang et al., 2018; Pisu et al., 2020). As reviewed by Vanden Driessche et al. (2013), the neonate/infant susceptibility toward tuberculosis might be due to hypo- or distinct responsiveness and limited anti-microbial activity of macrophages and neutrophils (Goenka et al., 2020; Shey et al., 2014). To explore these possibilities in *X. laevis*, we used a tagged recombinant *X. laevis* Csf1 to label a subset of CSF1R+ macrophages in combination with DsRed + *M. marinum* to determine by flow cytometry pathogen loads in peritoneal myeloid cells of tadpoles and adult *X. laevis.* The use of labelled recombinant ligand such as Csf1 or chemokine is commonly used as surrogate of specific antibody to investigate the cellular distribution of their respective receptors (Csf1r) (Samanta et al., 2021; Wu et al., 2021). Our results showed than upon intraperitoneal inoculation, DsRed + *M. marinum* is detected in peritoneal Csf1r + macrophages and Csf1r-granulocytes of both tadpoles and adults (Fig. 1A). This result suggests that similar to mammals, macrophages and neutrophils are involved both in early immune responses and in immune evasion (Lovewell et al., 2021, reviewed in Huang et al., 2018). Infected myeloid cells from tadpoles show a higher DsRed Mean Fluorescence Intensity (MFI) signal than their adult counterparts, which suggests that X. laevis tadpoles myeloid cells are more permissive and/or elicit lower antimicrobial activity toward M. marinum".

Fluorescence Intensity (MFI) signal than their adult counterparts, which suggests that *X. laevis* tadpoles' myeloid cells are more permissive and/or elicit lower antimicrobial activity toward *M. marinum* (Fig. 1B). Importantly, both in tadpoles and adult frogs, the DsRed MFI significantly decreased overtime (Fig. 1B). These results reinforce previous work showing that tadpoles are immunocompetent and develop a delayed immune response (Chen and Robert, 2011). The hypo- and distinct responsiveness leading to the permissiveness of larval frogs and human infants toward mycobacteria could be related to intrinsic features of the larval/fetal-derived myeloid cells as suggested *in vitro* with human macrophages (Goenka et al., 2020; Ó Maoldomhnaigh et al., 2021; Shey et al., 2014). In agreement with this view, a subpopulation of tadpole-specific myeloid cells in the kidney of *X. laevis* have recently been proposed to be responsible of the lower ranavirus load observed in tadpoles (Kalia et al., 2022). An alternative and non-exclusive possibility is that the larval/fetal tissue microenvironment may have immunoregulatory activity on innate immune cells to prevent a potentially deleterious tissue inflammatory response (Bain and MacDonald, 2022; Papaioannou et al., 2021).

b. Potential persistence of larval T cells in adults and relevance for adult immune response

As reviewed by Rudd (2020), increasing evidence suggests that mammalian fetal-derived T cells represent a distinct lineage from their adult counterparts like fetal-derived B1 B cells and adult-derived B2 B cells. In addition to representing a major component of the immune defense early in development, mammalian fetal-derived T cells are maintained in adulthood where they preserve their unique function in self-tolerance and protection during infection (Reynaldi et al., 2019; Smith et al., 2018; Yang et al., 2015). As reviewed by Zhivaki and Lo-Man (2017) and Rackaityte and Halkias (2020), infants are also thought to have developed specific T cell memory responses after *in utero* exposure to viruses, *plasmodium,* and bacteria including *Mtb* (Malhotra et al., 1997; Mishra et al., 2021; Odorizzi et al., 2018). In *X. laevis,* it has been suggested that larval-derived T and B cell memory can persist through metamorphosis and could be important for the adult T cell mediated immune responses (reviewed in Flajnik et al., 1987; Rollins-Smith, 1998). Likewise, it has been reported that immunization of bullfrog tadpoles with adult-derived hemoglobulin prevented the development of adult globin after metamorphosis (Maniatis et al., 1969). As reviewed by Rollins-Smith (1998), immunization with T cell-dependent antigens, T cell-mediated allograft rejection, or T cell-dependent tumor rejection during the larval stage accelerated rejection in adult Anura and at a lesser extent in Urodela. In *X. laevis*, several groups have shown that MHC- or Minor Histocompatibility-disparate allografts are tolerated by tadpoles and metamorphic frogs, and adults frogs primed by these allografts develop a persisting active T cell-mediated tolerance (Barlow and Cohen, 1983; Ono and Tochinai, 1995; reviewed by Robert and Ohta, 2009; Rollins-Smith, 1998). This tolerance can be ablated by inhibiting T cells with cyclophosphamide or by eliminating T cells by thymectomy (Barlow and Cohen, 1983; Horton et al., 1989; Ono and Tochinai, 1995). Furthermore, immunization of tadpoles with *X. laevis* lymphoid tumor cells did not induce tolerance to skin grafts transplanted during adult life but rather accelerated their rejection (Robert et al., 1997), and priming with irradiated lymphoid tumor cells at tadpole stages partially protected young adult frogs challenged with live tumors (Robert et al., 1995). Finally, it has been shown using thymus transfers that tadpole-derived T cell persist after metamorphosis (Rollins-Smith and Davis, 1992; Turpen and Smith, 1989).

As previously mentioned, early developing MAIT cells in mice can highly impact the pathogen load by boosting the conventional T cell response in mice (Mayassi et al., 2021; Sakai et al., 2021). To date, it is unknown whether larval iT cells persist in adult frogs or differentiate

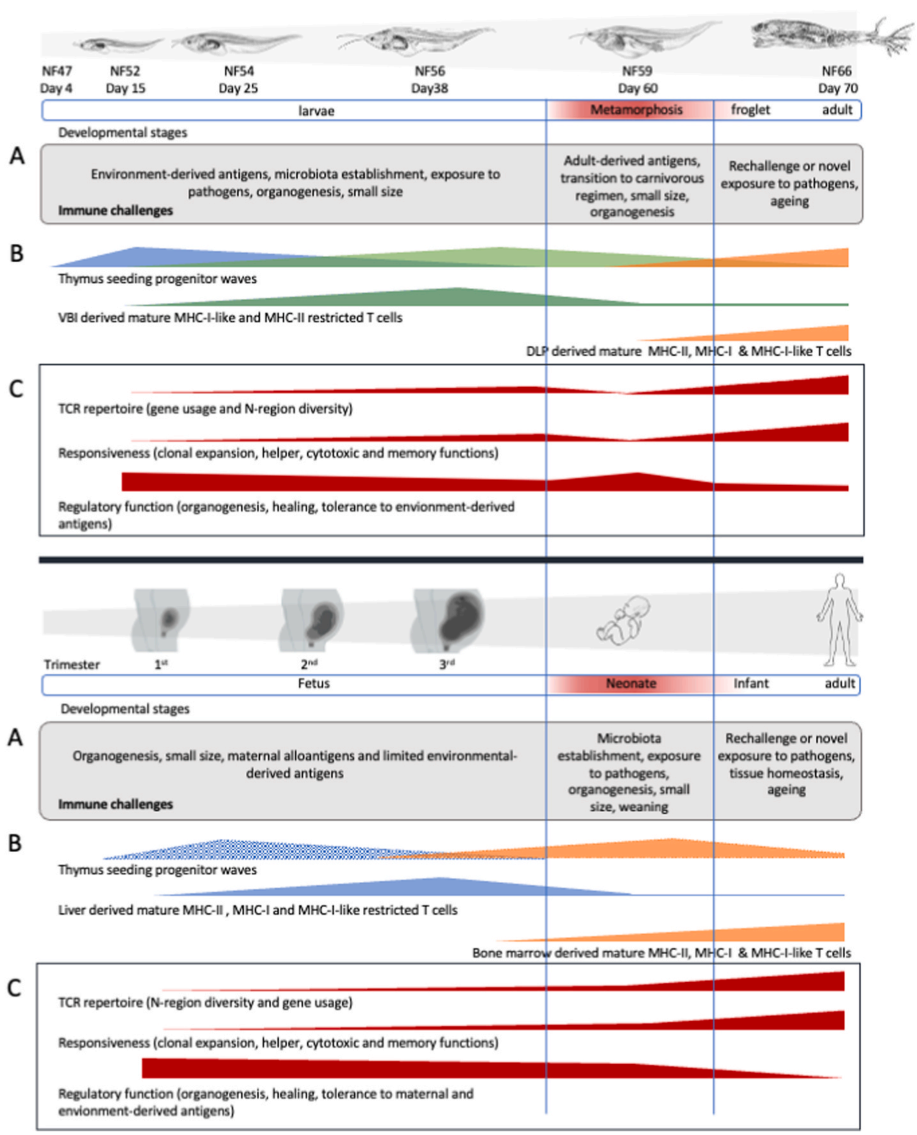

Fig. 2. Comparative overview of *X. laevis* and human T cell development (adapted from Davenport et al., 2020; Park et al., 2020; Robert and Ohta, 2009).

(**A**) During ontogenesis, *X. laevis* and humans encounter developmental stage- and species-specific immune challenges. Developmental processes such as organogenesis can be damaged by excessive inflammatory immune responses. In addition, *X. laevis* tadpoles are devoid of maternal protection.

(**B**) Larval/fetal T cells and adult T cells have distinct embryonic origins. In tadpoles, the thymus is colonized by two thymic seeding progenitor (TSP) waves from the ventral blood island. The first wave in blue starts at 4 days post-fertilization (stage 47) and the second one before day 33 (stage 55), which replaces thymocytes from the first wave by day 42 (stage 57). In *X. laevis*, mature peripheral T cells are detected at day 15 post-fertilization (stage 52) (represented as a merged green color of the two TSP waves because their respective contribution in peripheral T cells is unknown). The maximum larval splenic T cell number is reached at day 44 (stage 58). A third TSP wave originating from dorsolateral plate (DLP) starts during metamorphosis (orange) and gives rise to adult-type peripheral and conventional T cells (orange). In humans, two distinct TSP waves have been suggested. The first TSP wave coming from the liver starts at 8 weeks post-conception (in dashed blue) and gives rise to peripheral T cells that are first detected at 10–11 weeks post-conception (blue). A second wave coming from the bone marrow probably starts before birth (orange) and gives rise to the peripheral adult-type conventional T cells (orange).

(**C**) Traces in red depict the relative feature changes in the peripheral T cells. The TCR repertoire and responsiveness increase during the development, while the regulatory function decreases. Of note, during metamorphosis the number of peripheral T cells drops and the regulatory function increases to face the stage-specific immune challenges (see text for details).

from new precursors leading to adult-type iT cells. Although, reverse genetics has shown that MHC1b-UBA4 interacting iVα45 positive cells are critically involved in the tadpole anti-mycobacterial response, their role in the adult remains elusive (Rhoo et al., 2019). In mammals, early developing MAIT cells can impact adult anti-mycobacterial immune defenses (Sakai et al., 2021). Thus, it is possible that early developing T cell-mediated immune defenses in tadpoles can interfere with the outcome of the mycobacterial disease in adult frogs as well as in mammals.

c. Is larval and adult immune cell function imprinted by their precursor origin?

The intrinsic differences of T cells between mammalian fetal and adult stages is postulated to be due to the distinct developmental origin of their progenitors (Davenport et al., 2020; Mold et al., 2010). As reviewed by Ciau-Uitz et al. (2010) and more recently by Elsaid et al. (2020), mammalian, bird, *Xenopus,* and fish blood cells derive from three orchestrated waves of hematopoietic progenitors of different origins during embryogenesis, which sustain hematopoiesis throughout the lifetime (Ciau-Uitz et al., 2000; Turpen and Smith, 1989). In mammals, primitive hematopoiesis is initiated in the extra-embryonic yolk sac and rapidly seeds the liver. This is followed by a second wave of progenitors from the extra-embryonic yolk sac that provides definitive hematopoiesis. The precursors expand in the liver before migrating to the bone marrow (Bennett and Bennett, 2020; Feyaerts et al., 2022; Lavin et al., 2015). In *Xenopus* and axolotl, the liver most likely represents the main hematopoietic organ throughout life (Debuque et al., 2021; Elsaid et al., 2020). For the thymus, precursors migrate in two waves in mice and zebrafish, whereas three successive waves have been identified in *Xenopus* as in birds (Ciau-Uitz et al., 2010). In *Xenopus,* the third wave of stem cell migration into the thymus occurs after metamorphosis (Rollins-Smith and Davis, 1992; Turpen and Smith, 1989). Thus, as in mammals, the functional and phenotypical differences between tadpole and adult T cells are likely intrinsic and related to their distinct embryonic origins (Mold et al., 2010).

In mammals, tissue resident macrophages (TRM) originally arise during embryogenesis from the yolk sac. Over the life course, TRMs are replenished from liver and bone marrow-derived macrophages depending on their subtypes (*e.g.,* microglia *vs.* alveolar macrophages) or following infection or disease. So far only the microglia have been detected with a phenotype that is impacted by their developmental origin (*i.e.*, liver *vs.* bone marrow; Bain and MacDonald, 2022; Bennett and Bennett, 2020). In contrast to neutrophils and dendritic cells from secondary lymphoid organs that are considered to be short-lived circulating immune cells, TRMs are self-renewing macrophages that can persist throughout life (Liu et al., 2007; reviewed in Patel et al., 2021). TRMs are important in local immune surveillance, remodeling, homeostasis and specific functions based on their tissue residency, (*e.g.,* alveolar macrophages in the lung, osteoclasts in the bones and microglia in the brain; reviewed in Bain and MacDonald, 2022; Bennett and Bennett, 2020; Murray and Wynn, 2011). In non-mammalian species including *Xenopus* and axolotl, ontogenesis, and lifespan of TRMs remains largely unknown and may differ from mammals. Contrasting with mammals, embryonic microglia in zebrafish are replaced by a second wave of embryonic monocytes in the absence of injury or disease (Ferrero et al., 2018). After metamorphosis in *Xenopus*, the bone marrow is formed where the late stage of myelopoiesis takes place (Grayfer and Robert, 2013; Yaparla et al., 2020). In Urodela, the bone marrow is not involved in myelopoiesis, which probably only takes place in the liver throughout the life (Debuque et al., 2021). In fact, liver-derived macrophages play a major role in organ regeneration in axolotls (Debuque et al., 2021). While the importance of ontogenetic imprinting remains to be fully evaluated, it is clear that this will need to be combined with tissue microenvironments to acquire mature resident identity. This undeniably represents a major factor influencing macrophage phenotypes and function (Bain and MacDonald, 2022), as we are reminded by the evolutionarily conserved plasticity of macrophages and monocytes in vertebrates (Edholm et al., 2017; Grayfer et al., 2018).

5. Conclusion and perspectives

Current knowledge on amphibian immunology suggests that like in the mammalian fetus, amphibian larval T cells and myeloid cells are distinct phenotypically and functionally from their adult counterparts (Fig. 2 for T cells). Both developing amphibian tadpole- and mammalian fetal-derived cells cells must be able to elicit immune defenses against prevalent pathogens, prevent harmful inflammation, maintain tolerance toward environmentally-derived antigens, promote organogenesis and establish tissue resident immune populations (Al Nabhani and Eberl, 2020; Constantinides et al., 2019; Davenport et al., 2020; Feyaerts et al., 2022; Olszak et al., 2012). In contrast to free-living amphibian larval T cells, human fetal T cell-mediated pro-inflammation/tolerance will be solicited only after birth and during weaning stages when the developing mammals is devoid of the mother's protection (Al Nabhani et al., 2019; Al Nabhani and Eberl, 2020; Feyaerts et al., 2022). The small number of T cells at these early life stages appears to result in a narrower TCR repertoire (segment usage and N-region diversity) favoring thymic positive selection, detection of conserved antigens related to tissue homeostasis, avoiding strong microbiota- and self-reactive T cell inflammatory response. Evidence suggests that in *X. laevis* some T cell populations persist in adult amphibians and may play a role in the host-mycobacteria interaction as recently observed in mice with MAIT cells (Sakai et al., 2021). As such, free-living amphibian larvae represent a model of choice to investigate (1) early developing immune cell regulatory mechanisms in tetrapods involved in immune tolerance, tissue homeostasis, and host pathogen interaction; and (2) the interaction between early and late developing immune cells. *X. laevis* is also an ideal model to unravel potential developmental immunotoxic effects of pollutants which are irreversible or long lasting (Robert et al., 2019), reviewed in (Nagel et al., 2020). Advanced genome editing techniques (CRISPR/Cas9, siRNA) are being increasingly used to investigate MHC Class Ib restricted iT cells in *Xenopus* (Edholm et al., 2013, 2018). In mammals, the molecular mechanisms regulating fetal immunity and their role in the adult host-mycobacterial interaction remains largely understudied. Improving our knowledge of larval/fetal T cells and myeloid cells is crucial to design new vaccines for protecting animal and neonates/infants from mycobacteriosis as well as against other infectious diseases. In fact, the neonates and infants are relatively unresponsive to current vaccines (Zhang et al., 2017).

Data availability

Data will be made available on request.

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