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What can we learn about fish neutrophil and macrophage response to immune challenge from studies in zebrafish

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A B S T R A C T

Fish rely, to a high degree, on the innate immune system to protect them against the constant exposure to potential pathogenic invasion from the surrounding water during homeostasis and injury. Zebrafish larvae have emerged as an outstanding model organism for immunity. The cellular component of zebrafish innate immunity is similar to the mammalian innate immune system and has a high degree of sophistication due to the needs of living in an aquatic environment from early embryonic stages of life. Innate immune cells (leukocytes), including neutrophils and macrophages, have major roles in protecting zebrafish against pathogens, as well as being essential for proper wound healing and regeneration. Zebrafish larvae are visually transparent, with unprecedented *in vivo* microscopy opportunities that, in combination with transgenic immune reporter lines, have permitted visualisation of the functions of these cells when zebrafish are exposed to bacterial, viral and parasitic infections, as well as during injury and healing. Recent findings indicate that leukocytes are even more complex than previously anticipated and are essential for inflammation, infection control, and subsequent wound healing and regeneration.

1. Introduction

Most fish live throughout life in water, in which they are constantly in close contact with potential pathogens. One millilitre of sea water could contain 10 million viruses, one million bacteria and about 1000 small protozoans and algae [1], and some of these are harmful for fish. Similarly, zebrafish are exposed to a range of pathogens in their freshwater habitats, including rice fields of South Asia, before they develop a mature adaptive immune system 4 weeks after hatching [2,3]. Therefore, a system to constantly survey potential dangers is essential. This system must be able to immediately combat dangerous organisms as well as send signals to other compartments in the body, to enhance systemic responses. This system is called the innate immune system and is found with different degrees of sophistication in all animals [4,5].

Teleosts (bony fish), which represent half of all vertebrate species, diverged evolutionarily from mammals 450 million years ago [6] and today we know of more than 33,000 different species, which makes it the most diverse group of vertebrates [7]. Following the divergence, a whole genome duplication took place in fish in the Mesozoic era (252-66 million years ago) [8]. Gene duplications have also occurred, dramatically increasing the number of genes including those in pathogen recognition (eg, tol-like receptors, TLRs). Therefore, fish may have a more diverse innate immune system compared to mammals, who rely to a higher degree on the adaptive arm of the immune system [6,9]. This frames the innate immune system as an essential mechanism for fish and highlights the importance of understanding the different components, pathways and processes. In this review, we will focus on cells of the innate immune system and more specifically on two types of leukocytes: neutrophils and macrophages. These cells are professional phagocytes and have key roles in combatting pathogens, signalling to the rest of the immune system, repairing wounds, and regenerating tissue [10].

Neutrophils and macrophages have pattern recognition receptors (PRRs) on their cell surface [10,11]. These receptors recognise pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) and when activated, a cascade of reactions is initiated leading to (mostly) appropriate responses [12]. The nature of the response depends on whether bacteria, virus, parasites or an injury is the cause of activation [12]. Obtaining detailed knowledge on these mechanisms as well as understanding the difference from mammals improves our understanding of immunological reactions and evolution of the immune system. Furthermore, this knowledge contributes to the development of strategies to manage fish disease in the fisheries/production systems with regard to improvement of prophylaxis and control measurements.

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The zebrafish has been used as a model organism since the 1980s when George Streisinger fought for people's acceptance of the value of this model [13]. As with many other pioneers, he died before the recognition of the usefulness of the model impacted the scientific world and since the 1990s the number of zebrafish publications has increased dramatically (Fig. 1).

The zebrafish larval model, combined with transgenic lines labelling immune cell populations [14,15], has allowed high resolution 4D microscopy, which has shed light on the complexity of the neutrophil and macrophage response to wounding and infection in fish. However, zebrafish are a single species and, much like using murine models for human disease where there are important differences in immune responses, care must be taken when extrapolating findings to other species due to the huge diversity between fish species.

2. Fish innate immune systems

Fish have both innate and adaptive immunity but rely more on innate immunity to maintain homeostasis compared to higher vertebrates due their constant close contact with pathogens through the environment and their exposure to pathogens from the early embryonic stage [9]. Fish possess primary and secondary lymphoid organs as higher vertebrates, except that they lack classical bone marrow and lymph nodes [9,16]. The functions of these organs are therefore covered by other tissues such as the head kidney and spleen [17]. They do, however, have melano-macrophage centers and rodlet cells not encountered in higher vertebrates [16]. They are a diverse group of animals, which is reflected in the different composition and mechanisms of the immune system. A fascinating example of this diversity is found in the Atlantic cod, which lacks the major histocompatibility complex II (Mhc II), a protein believed to be crucial for adaptive immunity and survival from disease [18]. Despite the lack of Mhc II, the fish survive perfectly well. It has been hypothesised that some of the Mhc I gene copies they possess have evolved to include some of the functional roles of Mhc II [19]. Fish have more genes represented in the innate immune system compared to mammals that have a higher complexity of their adaptive immune system [6].

The innate immune system is classically divided into three compartments: physical/surface barriers, cellular and humoral components [5,20]. It can be furthermore divided into constitutive and inducible, where the constitutive immune limiting response acts quickly with ligand binding to receptors and inducible acts slower but more strongly, sometimes causing immunopathology and tissue damage [4,21]. PRRs are an essential part of the innate responses and amongst them are toll like receptors (TLRs) which are found both on neutrophils, macrophages and other innate immune cells. PRRs recognise PAMPs and DAMPs and upon activation, an inflammatory response is initiated. Adaptive immunity and antibody production is dependent on direction from the innate immune system [21]. The adaptive immune system may in some fish species play a minor role, which was demonstrated in a study where inhibition of the adaptive arm of the immune system (B and T cells) in *rag*$^{-/-}$ zebrafish did not result in decreased protection following re-infection with a bacterium [22]. The Atlantic cod is another example of a fish species that may rely more on innate functions. It lacks Mhc II but has many more genes for TLRs [18,23]. It is known to have a poor antibody response [23,24] but is capable of producing specific immunoglobulins probably with cross presentation from Mhc I [19].

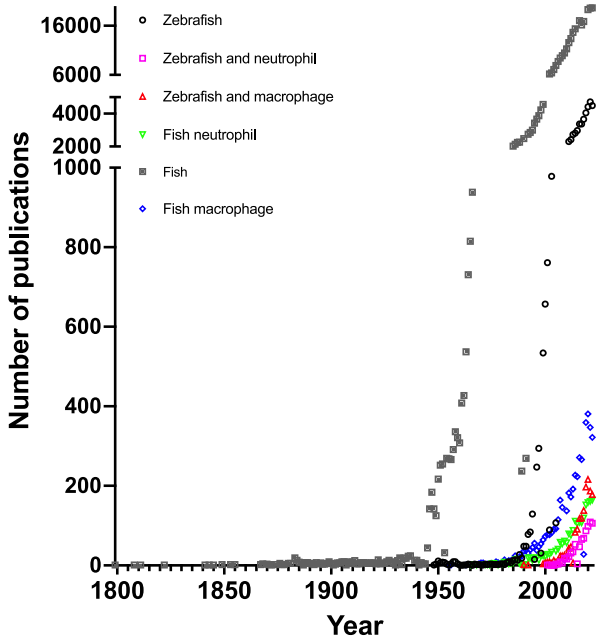

Fig. 1. Using Pubmed the number of publications is depicted showing the result of searches with the words "*fish*", "*zebrafish*", "*fish macrophages*", "*zebrafish macrophages*", "*fish neutrophils*", "*zebrafish neutrophils*" over the years.

Neutrophils and macrophages are professional phagocytes meaning that they are efficient ingesters of microorganisms and cell debris upon recognition by receptors [25,26]. Macrophages are also professional antigen presenting cells (APCs) and present antigens to T cells on MHC II molecules. Other cell types are furthermore important in innate responses such as granulocytes, red blood cells, thrombocytes, B cells and subtypes of T cells [21].

Fish mucosal surfaces such as skin, fins, gills, intestine, nasal cavity and the newly described Nemausean lymphoid organ (NELO) [27] are immunologically active barriers representing the first line of defence in protection from various insults such as pathogen attack. These surfaces are covered in mucus, which has antimicrobial properties, and are inhabited by beneficial microorganisms (called the microbiome). Therefore, it is key to be able to differentiate "friend" from "foe". Neutrophils and macrophages are essential players in this differentiation. The cells are activated by, amongst other things, complement factors, which are components of the mucosal barriers and are activated by pathogens. A study by Earley et al. (2018) showed that complement genes were down-regulated and that the composition of the microbiome in the intestine changed with the depletion of interferon regulatory factor 8 (Irf8) dependent macrophages, illustrating the impact macrophages may have on homeostasis of the fish [28]. Keratocytes, which are found in the outer epidermal layer, are also phagocytotic and may play important roles in pathogen phagocytosis before dying and being sloughed off from the epidermis [21].

The function of the complement system in an immunological reaction is opsonisation, inflammation and killing of pathogens with the formation of a membrane attack complex [29,30]. The system consists of plasma proteins primarily produced in the liver reacting in a cascade manner with direct or indirect killing of pathogens as a consequence. The system can be activated in three different ways: 1) classical (activated by antibody-antigen complexes), 2) lectin (activated by lectins) and 3) alternative pathway (activated by lipopolysaccharides present on some pathogen surfaces) [29,30]. The complement system is important for the activation of neutrophils and macrophages. Activation of the system can result in attraction of neutrophils and macrophages and stimulate phagocytosis and can also induce inflammation [29,30]. This system is a good example of the expanded use of innate responses in fish compared to mammals, since many of the complement factors have diversified into different isoforms with functional diversification as a result [31]. Many genes of the complement system in mammals have homologs in teleosts [31]. Activation of complement factor C5a (chemoattractant) in the classically activated and alternative pathway activates macrophages and neutrophils [32]. C3b is the most important factor for opsonizing pathogens, which stimulates neutrophils and macrophages to phagocytose the opsonized objects [33,34].

More information on other components of the innate immune system

can be found in the rest of this special issue and in e.g. Dalmo et al. (2022) [21].

3. Zebrafish neutrophils

3.1. Neutrophil development

Neutrophils are the primary white blood cell to respond to infection or injury in order to restore homeostasis within an organism [35]. Haematopoiesis is the development of all blood cells, including those of the myeloid and erythroid lineages, and this process is conserved between vertebrates [36–39]. In zebrafish and other teleosts, neutrophils develop through two distinct waves of haematopoiesis (Fig. 2); primitive haematopoiesis, around 24 h post fertilisation (hpf) [40], develops spatially distinct populations of myeloid (neutrophil, mast cell, and macrophage) and erythroid cells, with this then followed by a definitive wave of haematopoiesis, at 48-72hpf, which produces all sub-types of blood cells, including lymphoid cells [41]. During the initial phase in fish, primitive neutrophils are produced in the rostral blood island (RBI) [25,37,42,43] and are believed to be immature neutrophils due to the lack of granule Sudan Black staining, an indication of neutrophil maturation [44]. A transient wave of erythromyeloid progenitor-derived neutrophils are subsequently formed in the posterior blood island (PBI) [45], which further develops into the caudal haematopoietic tissue (CHT). Haematopoietic stem cells migrate to the CHT and mature neutrophils that stain positive for both neutrophil and granule markers, myeloid peroxidase and Sudan Black, develop from this population [44,46].

3.1.1. Neutrophil maturation

As the first recognisable cells of the neutrophil lineage (myeloblasts) mature, they sequentially develop different granules, in a process known as granulopoiesis [47]. These granules are essential for the successful function of neutrophils during an inflammatory response [48]. Primitive neutrophils are not classed as mature neutrophils due to the lack of granule markers. However, neutrophils derived from haematopoietic stem cells (HSC) during the definitive wave of haematopoiesis express many granule types [49,50]. There are three categories of granules; primary (azurophilic) granules containing myeloperoxidase, lysozyme and elastases, secondary (specific) granules containing matrix metalloproteinases such as collagenase, and tertiary granules containing gelatinase. Human neutrophil maturation during granulopoiesis is well documented [50,51], however, less is documented about the individual stages of zebrafish neutrophil development and there are some differences. The early zebrafish myeloblast becomes the promyelocyte, similar to human neutrophil progression. It has a rounded nucleus but lacks the coarse, azurophilic primary granules characteristic of human promyelocytes. As neutrophil maturation progresses, the cells become smaller in size, the chromatin condenses and divides into lobes, and the cytoplasm acquires the characteristic granules of mature neutrophils [52]. The adult zebrafish kidney and spleen contain neutrophils at various stages of development, including promyelocytes with a large, round nucleus and diffuse nuclear chromatin, and maturing neutrophils that have segmented nuclei with two or three lobes [52]. In larval zebrafish, most neutrophils have a kidney-shaped nucleus, and about 15–25% have a bilobed nucleus, but only rarely have a multilobed nucleus [44,53–56].

As neutrophils mature during larval stages into adulthood, their nuclear envelope changes in morphology [57]. Zebrafish neutrophils contain a polymorphic nucleus [55,57] as seen in mammals, such as mice and humans. Like their human counterparts, zebrafish neutrophils have segmented nuclei; however, their nuclei are divided into two or three lobes instead of the five lobes typically found in human neutrophils [52].

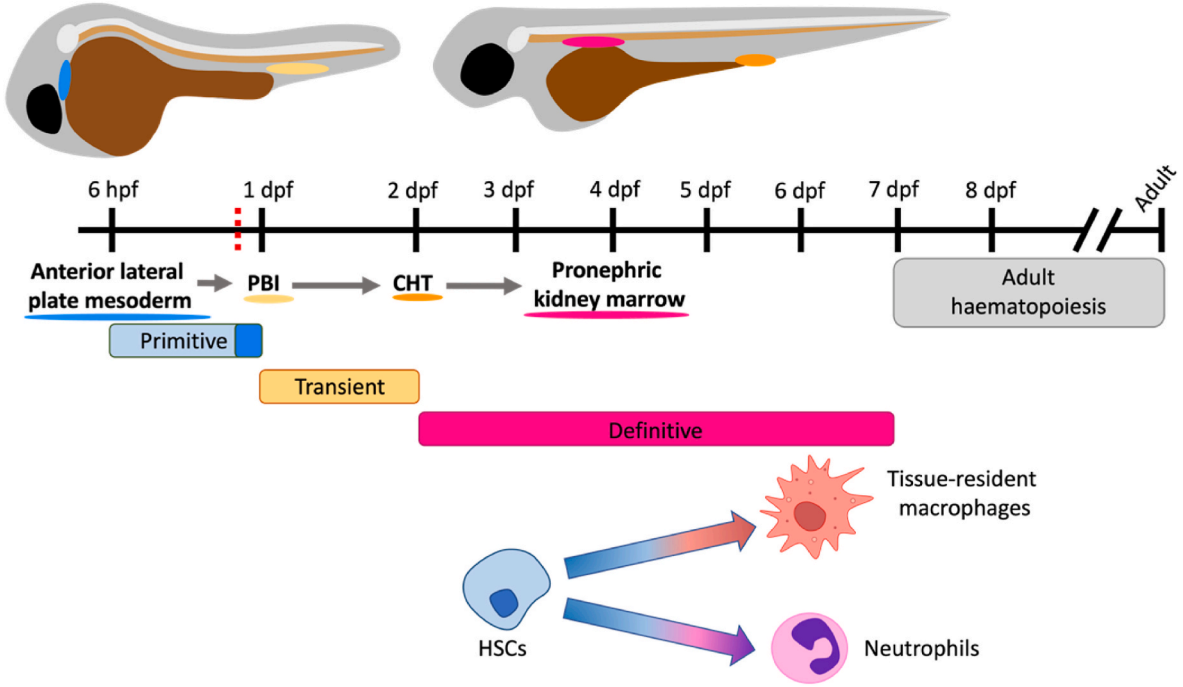

Fig. 2. Summary of neutrophil and macrophage ontogeny in zebrafish. The anterior lateral plate mesoderm (blue) develops at 6 hpf, where myeloid cell precursors originate. At 20–22 hpf, the primitive wave of haematopoiesis occurs. From 24 hpf and onset of circulation, the PBI (yellow) develops, and the transient wave of haematopoiesis begins. The PBI then transitions into the CHT (orange) at 2 dpf, marking the start of the definitive wave of haematopoiesis, with HSCs differentiating into tissue-resident macrophages and mature neutrophils. From 4 dpf, the pronephric kidney marrow (pink) begins to develop, resulting in a gradual replacement of the embryonic haematopoietic system and onset of adult haematopoiesis at 7 dpf. Abbreviations: hpf, hours post-fertilisation; dpf, days post-fertilisation; PBI, posterior blood island; CHT, caudal haematopoietic tissue; HSCs, haematopoietic stem cells.

3.1.2. Neutrophil production and lifespan

In mammals, neutrophils are found predominantly in the circulation, waiting for inflammatory cues. In humans, neutrophils make up around 40–70% of circulating leukocytes [51]. Zebrafish and other teleosts differ, as fewer than 5% of neutrophils are in the circulation [25,44,58–61]. During larval stages, zebrafish neutrophils reside throughout the body within tissues and in the CHT until the kidney develops [25,62–64] - this is the site of granulopoiesis in adulthood, ready for deployment of neutrophils to an inflammatory stimulus. The lifespan of neutrophils can vary depending on the environment in which they find themselves and is difficult to study due to their very short lifespan once removed from tissue microenvironments in e.g. *ex vivo* experimentation. Transparent zebrafish larvae are a tractable model to investigate neutrophil lifespan *in vivo*, with one study in the zebrafish [65] measuring the half-life of tissue neutrophils in 3 days post fertilisation larval zebrafish to be 5 days. These neutrophils are likely to be both primitive and definitive but there is no evidence to suggest that they differ in function. In mammals, circulating neutrophils are documented as having a half-life of 6–12 h [66,67], although it has been suggested that it may be more similar to that measured *in vivo* in zebrafish, around 5 days [68]. This lifespan can be significantly altered due to an inflammatory stimulus and remains controversial due to a lack of *in vivo* models where these studies are possible.

3.2. Neutrophil responses to immune challenge

It is well established that following an inflammatory stimulus, neutrophils become primed and activated, ready to respond to invading pathogens [69–71]. One of the first responses of the neutrophil following stimulation, is movement out of the circulation or through tissues using adhesion molecules [69,70]. This process is largely dependent on CD11/CD18 activation [72]. Any tissue damage, be it caused by a mechanical wound or invading pathogen, triggers an increase in hydrogen peroxide (H_2O_2) [73] at the damaged site, to which the activated neutrophil can respond. A hydrogen peroxide gradient forms, emanating from the wound site, which is thought to be the primary signal for neutrophils to begin migrating to the wound in zebrafish. It has been identified that the Src family kinase Lyn acts as an endogenous H_2O_2 sensor that initiates neutrophil recruitment to the wound in zebrafish larvae [74]. Other signals are also generated following tissue damage or infection which facilitate the migration of neutrophils through tissues. DAMPs, including DNA, proteins, extracellular membrane components [75] as well as chemokines such as Cxcl1 and Cxcl2 [76,77] are detected by neutrophils, promoting further neutrophil recruitment.

As mentioned previously, H_2O_2 and other signals such as DAMPs promote neutrophil migration through the tissue to an area damaged by mechanical injury or pathogen invasion. Zebrafish, just like in humans, have an initiation phase of inflammation that is characterised by the increased accumulation of neutrophils to a focal point which peaks at around 4–6 h post injury or infection [14,78]. The migration of neutrophils to sites of challenge in zebrafish includes a behaviour known as neutrophil swarming, first observed in murine models of inflammation [79–82]. Swarming is defined as the gradual accumulation and cluster formation in a highly coordinated manner of chemotaxis following injury [79] or infection [80]. Lämmermann et al. (2013) showed that leukotriene B4 (LTB4) is a key driver of neutrophil swarming acting as a relay signal [81]. Since then, swarming has been identified in zebrafish following tailfin injury or infection [79,80] with a role of LTB4 conserved [79,80]. Swarming has been observed in sterile tail-fin amputation [79] and in otic vesicle infection [79]. In tailfin transection it was shown that neutrophils form a swarm around a "pioneer" neutrophil that is often the first neutrophil to reach the tailfin wound. Pioneer neutrophils undergo a form of lytic cell death that resembles neutrophil extracellular trap (NET) formation. Neutrophils respond to bacteria injected into the otic vesicle, with this recruitment being amplified by a second wave of recruitment and dense clusters being formed [79], another hallmark of the swarming process. Poplimont (2020) has shown that a cell contact-dependant mechanism through calcium alarm signals enhances swarming and aids bacterial clearance, however the full molecular mechanism and *in vivo* purpose of swarming remains unclear [80].

3.2.1. Neutrophil function at inflammatory sites

3.2.1.1. Phagocytosis. Once recruited to sites of inflammation or infection, neutrophils can perform a variety of roles to combat pathogens and/or remove cell debris caused by tissue injury. One key mechanism is through phagocytosis of the pathogen or damaged cells to remove it from the host tissue and prevent further tissue damage through necrosis or dissemination of the pathogen. Zebrafish have been used to investigate phagocytosis extensively using a wide variety of bacterial and fungal pathogens, including *Pseudomonas aeruginosa*, *Staphylococcus aureus, Penicillium marneffei*, at different infection sites such as Duct of Cuvier, caudal vein or somatic muscle [15,83,84]. Various transgenic fluorescent reporter lines, such as *Tg(mpx:GFP)i114* to study neutrophils and *Tg(mpeg:mCherry-CAAX)sh378* to study macrophages [85,86] have allowed visualisation of bacterial engulfment and acidification of phagosomes in live infected zebrafish larvae. Neutrophils predominantly phagocytose bacteria present in tissues, rather than systemic infections in the bloodstream, where phagocytosis is often performed by macrophages [87]. *Staphylococcus aureus* is a common opportunistic pathogen that is phagocytosed by neutrophils; however, the bacteria utilise the host autophagy machinery to evade intracellular killing mechanisms [88]. The neutrophil can provide an intracellular niche for bacterial dissemination rather than bacterial killing [84,88]. Often, the type of infection and subsequent phagocytosis, can determine whether this is beneficial or detrimental to the host.

3.2.1.2. NETs. Neutrophil extracellular trap (NET) formation is a mechanism by which neutrophils are able to capture pathogens and facilitate their destruction [66,89]. In addition to DNA and histones [89], NETs contain proteins from azurophilic (primary) granules such as neutrophil elastase, cathepsin G, and myeloperoxidase, as well as proteins from specific (secondary) granules and tertiary granules, such as lactoferrin and gelatinase. This killing process has been observed and studied using mammalian neutrophils *in vitro* [90–93], however *in vivo* models to visualise NETs are less readily available, with very few murine or fish models able to observe this process.

Palic et al. (2007) were the first to demonstrate NET formation from zebrafish whole kidney assays, as well as in the adult fathead minnow, another teleost family member [94,95]. They identified extracellular DNA and localisation of myeloperoxidase following stimulation in neutrophil cell suspensions. More recently, models using zebrafish larvae have identified *in vivo* NET release following exposure to wounding and bacterial or fungal infection [79,96,97]. In larvae, following tailfin injury, NET release was identified in pioneer neutrophils during the swarming response [79]. In the context of infection, there is evidence that NET release may be linked to neutrophil pyroptosis, a regulated pro-inflammatory mechanism of cell death. Pyroptosis, where the formation of plasma membrane pores results in cell explosion, and NETosis share common pathways and both processes can be activated within neutrophils following infection, enhancing the efficiency of clearing bacterial or fungal pathogens in zebrafish larvae [96,97]. The release of NETs, or NETosis, is traditionally thought to be a form of cell death, however there are now studies that suggest viable NET release occurs, with neutrophils still able to phagocytose once NETs have been released [98,99], however this is yet to be confirmed in zebrafish models. The occurrence of this event is infrequent and difficult to capture in live fish, therefore better tools and transgenics need to be developed to investigate real-time NET release during an inflammatory

response.

3.2.1.3. Degranulation. Degranulation is one of the mechanisms by which neutrophils are able to release anti-microbial components [48] in the extracellular matrix, via NET release or into the phagosome following phagocytosis of bacteria. The different types of granules within neutrophils have a variety of antimicrobial substances that can be released [100]. There are very few zebrafish models that look at neutrophil degranulation, and those that do are primarily based on *ex vivo* whole adult kidney assays [94]. Myeloperoxidase release, an indicator of primary granule degranulation, can be detected from whole zebrafish kidneys following stimulation with inflammatory mediators in an *in vitro* setting, therefore provides useful, yet limited information. However, tools are developing to investigate this process using whole larvae imaging, including fluorescent probes and transgenic lines [101,102]. Using fluorescent probes, granule release can be detected following phagocytosis of zymosan particles, accumulating in the phagosome, which is highly similar to human neutrophil degranulation. But determining which specific subtypes of granules are involved in neutrophil degranulation still warrants further study as these probes do not target myeloperoxidase-positive primary granules. Transgenic reporters, such as *Tg(lyz:Hsa.MPO-mEmerald,cmlc2:EGFP)sh496* [102], may prove useful in studying neutrophil granule dynamics *in vivo*. One study suggests that bacterial contact is not essential for degranulation in neutrophils to occur [103]. Two days post notochord infection with *E. coli*, and long after bacterial clearance, further neutrophils are recruited late to the site of infection. These myeloperoxidase-negative neutrophils accumulate along the notochord, and along with extensive tissue damage, this indicates that degranulation has occurred despite no direct contact or engulfment of bacteria. These differences indicate further research is required to fully investigate degranulation in zebrafish during an infection or inflammatory response.

3.2.2. Resolution of inflammation

The process of inflammation is essential to protect organisms from infection and tissue damage, both of which can lead to severe illness, surgery or even death if left uncontrolled [104]. Inflammation itself, however, is a process that can also be uncontrolled and cause tissue damage and disease. In 1982, Metchnikoff identified the beneficial aspects of inflammation and highlighted the importance of neutrophils and macrophages in the maintenance of tissue homeostasis (Discussed by Medzhitov, (2010) [105]). For successful inflammation resolution, neutrophils must be removed from the inflammatory site [106]. In zebrafish, key processes that assist in neutrophil removal, apoptosis [107] and reverse migration away from sites of inflammation [108], have been described.

3.2.2.1. Apoptosis. Apoptosis, or programmed cell death, is a key mechanism by which neutrophils die and are removed once they have completed their role at the inflammatory site. This process of neutrophil death and subsequent uptake by macrophages, or efferocytosis [109–111], was historically thought to be the only mechanism in humans by which inflammation resolved. However, zebrafish studies have shown that although neutrophil apoptosis and efferocytosis contribute to inflammation resolution, it is only a small proportion of neutrophils that are removed from inflammatory sites this way [112,113]. Nonetheless, this process can be manipulated in zebrafish to delay or promote neutrophil survival during the inflammatory response through manipulation of key genes in the apoptosis pathway, for example caspases [112,114], or survival factors, such as hypoxia inducible factors (Hif) [115–117]. Apoptosis can be easily detected within zebrafish using whole-mount antibody staining, such as TUNEL, anti-caspase 3 staining or through the use of apoptotic report lines, e.g. *Tg(mpx:FRET)sh237* [79,112,118–121]. These pathways are conserved between humans and zebrafish and therefore the study and manipulation of neutrophil apoptosis using zebrafish remains a very important area of research to opens up new opportunities for the development of novel therapeutics promoting inflammation resolution.

3.2.2.2. Reverse migration. Reverse migration is the movement of leukocytes away from a site of stimulation or in the opposite direction to the net leukocyte population [122]. The discovery of reverse migration of neutrophils was first identified in a zebrafish model [108,121] and since then, has been identified as a mechanism also applicable to murine and human neutrophils [123–126]. Wounding studies in larval zebrafish have previously shown that neutrophils primarily undergo reverse migration away from sites of inflammation as the key mechanism of inflammation resolution [108,127,128]. Following neutrophil recruitment to tailfin transection, reverse migration of neutrophils can be seen as early as 2–3 h post injury [108,120,121], prompting further studies identifying entry back into the circulation [108,129]. Interestingly, macrophages may also play a role in promoting the reverse migration of neutrophils [130,131]. Initially, it was thought that macrophage contact was necessary, however, several studies have shown neutrophils still exhibit reverse migration in the absence of macrophage contact. One study [130] found that 61.7% of neutrophils reverse migrated after direct contact with macrophages, however the remaining 'untouched' neutrophils were also able to reverse migrate. This was also confirmed in *irf8* morphants, where macrophage numbers are drastically reduced, where 35% of neutrophils reverse migrated. It has previously been shown that 71% of reverse migrated neutrophils did not encounter macrophages and 65% of the reverse migrated neutrophils did directly contact a macrophage [131]. These findings and others indicate that reverse migration of neutrophils can be influenced by cell-cell content [130], the release of soluble factors e.g PGE_2 and LXA_4 into the surrounding tissue environment [131], or changes in gene expression within neutrophils e.g. Hif expression [120], the Cxcl12/Cxcr4 axis [132] or the Cxcr2/Cxcl8 axis [133,134]. Furthermore, neutrophil phenotype before and after reverse migration (in terms of neutrophil responses and antibacterial effects) were not significantly different from neutrophils that had not been recruited to a wound site [128]. The optical clarity and advanced imaging techniques available in the zebrafish have allowed this process to be observed and studied in detail, however there is still much to learn about the mechanism, as well as the advantages or disadvantages reverse migration of neutrophils may have to the host. There are many studies that suggest there are different beneficial or detrimental effects of reverse migrated neutrophils. Neutrophil reverse migration can promote inflammation resolution locally by removing the neutrophils from the inflamed site but there is also the possibility of reverse migrating neutrophils entering the circulatory system, leading to the potential of systemic spread of inflammation or infection [135], however, this is yet to be determined in zebrafish models.

3.2.3. Neutrophil responses towards a fish parasite

The zebrafish has been used as a suitable model to investigate host-parasite interactions, focusing on neutrophil and to a lesser degree macrophage responses, when infected with the ciliated protozoan fish parasite *Ichthyophthirius multifiliis*. This parasite is an important pathogen and infects almost all species of freshwater fish on a worldwide scale and causes high morbidity and up to 100% mortality in fish production systems [136]. In the parasitic stage of its direct life cycle, it infects surface tissues such as the skin, gills and fins and settles above the basal lamina and is covered by one to a few fish cell layers. It creates an interstitial space in which it continuously rotates and moves around [137]. Here, it starts to feed from fish material and grows up to 1 mm in size becoming visible as a white spot to the naked eye – hence the name *white spot disease* [136,138,139]. Fish are able to acquire immunity against the parasite and therefore, a lot of research has gone into elucidating the immune responses responsible for protection [137,

139–158]. In immune fish, the parasites penetrate the surfaces but are prematurely forced to exit within a few hours. Responses of naïve and immunised fish have been compared and it is known that antibodies and a Th2-like response play a major role in adaptive immunity [139,146,147,159,160] but also complement [140,150,161] and cellular responses [148,156,162,163] play a part. An inflammatory response is dominant in naïve fish if they receive a high infection pressure [164], while more subtle infections induce more local responses. Neutrophils and macrophages have been described to be nearby the parasites and the interstitial space using histological techniques [162,165]. Due to the "snapshot" nature of histology analysis, the dynamic roles of these cells during parasitic infection were not established. They were never found touching the parasite and the immune cells were often found disintegrated [162,166]. Due to the nature of the infection under the surface of the skin, the infection is accessible to *in vivo* microscopy in most fish. Therefore, to increase the resolution of the roles of these cells, the infection has been applied to a zebrafish neutrophil reporter line, where host-parasite interactions directly can be observed. Adult zebrafish have a level of natural immunity towards the parasite, but persistent infection can be achieved when zebrafish are exposed to stressful conditions (e.g. overcrowding) [136]. In adult zebrafish, neutrophils were found to play a major role in both naïve and immune fish and were found accumulated at infection sites even after the parasites had prematurely exited the immune fish [148]. It was furthermore found that two days after infection, parasites in naïve fish ingested and neutralised neutrophils and thereby reduced immune responses both directly and indirectly by blocking the signals that the neutrophils were supposed to have sent to the rest of the immune system [148]. In zebrafish larvae, the behaviour of neutrophils, macrophages and the parasite have been studied [137]. The authors found that in early-stage infection, both neutrophils and macrophages often accumulated around the parasites (Fig. 3). Neutrophils were especially active in their migration around the interstitial space. The parasite rotated continuously, probably representing an immune evasive behaviour preventing innate immune cells from reaching and damaging the parasite. In the study, a timelapse video illustrated the death of a parasite, which may have been caused by neutrophil and macrophage attack [137]. Neutrophils exhibited balloon like structures, which may be an indication of NETs, when approaching the parasite. On a global scale, the gene for the macrophage marker *mpeg1.2* was found to be upregulated in larvae 8 h post infection further supporting their involvement in the disease. The authors discussed the different scenarios regarding the interaction of parasites and neutrophils and macrophages (Fig. 3). It was concluded that the neutrophil and macrophage response together with other innate mechanisms was critical to limit the severity of the disease demonstrating the importance of these cells during parasite attack.

The importance of innate immune cells in protection against *I. multifiliis* has also been reported in other fish species such as rainbow trout [163], carp [140,156] and channel catfish [167]. In carp, neutrophils were recorded as first responders to the infection and were followed by other cell types such as granulocytes, eosinophils and basophils [156]. In naïve carp parasite exit points were dominated by neutrophils whereas, in immune carp, more macrophages were found illustrating a switch in the response [162]. Besides directly killing the parasite as suggested in Mathiessen et al. (2023) [137], these cell types are also involved in various other immunological processes such as cytokine and chemokine production, phagocytosis, initiating the complement response, attraction, activation and migration of cells, all processes that aid fish in fighting the disease. In rainbow trout [163] and common carp [141] an increase in serum amyloid A (Saa) following infection has been reported. In mammals, SAA, which has chemoattractant properties towards cells such as T-lymphocytes and neutrophils [163], is synthesised by macrophages [168]. Saa have been found upregulated more than 1600 times in skin of carp following an *I. multifiliis* infection suggesting its involvement in the anti-parasitic response [141]. Furthermore, a downregulation of the neutrophil-expressed complement factor I in blood have been observed while an upregulation in skin was reported [140] suggesting the migration of neutrophils to the site of infection. This was also demonstrated in another study where C-lectin expression increased from blood

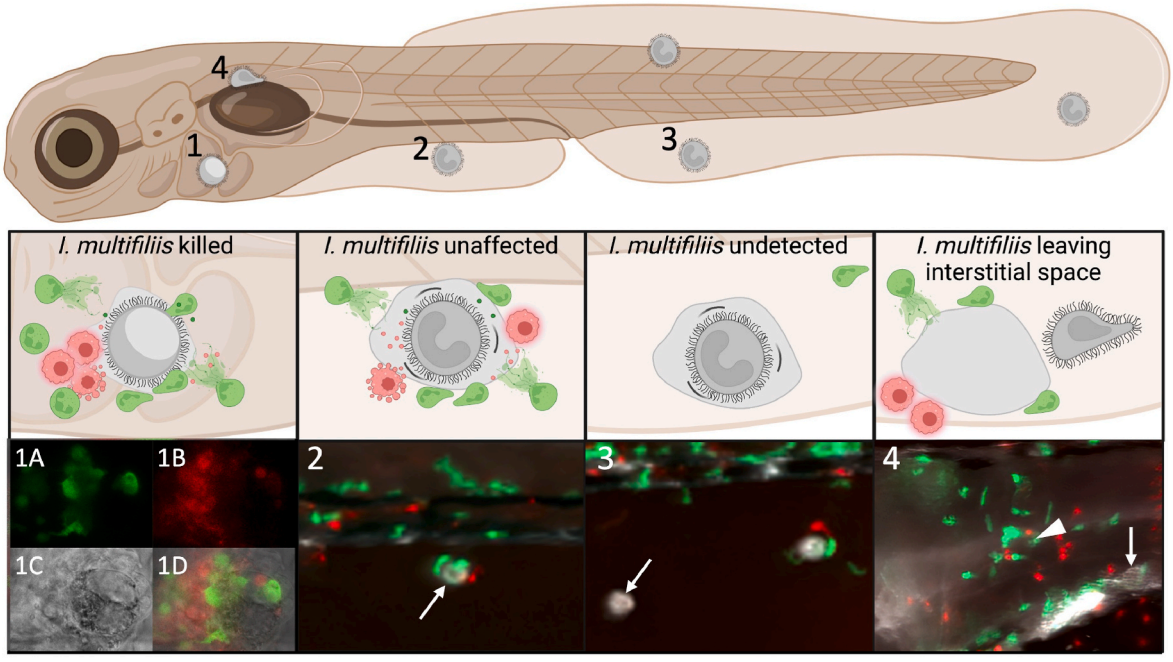

Fig. 3. Different scenarios observed in zebrafish larvae infected with the fish parasite *Ichthyophthirius multifiliis*. 1) Still image from a timelapse video taken with a confocal microscope from Mathiessen et al. (2023) [137]: Neutrophils (1A) and macrophages (1B) are surrounding a parasite, which just died seconds before (1C). 1D is a merge of 1A-C. Images 2–4 have been captured with a stereo microscope: 2) a parasite is surrounded by neutrophils and macrophages but was unaffected for the 5 h it was imaged (white arrow), 3) a parasite is not surrounded by phagocytes (white arrow) and 4) a parasite (white arrow) has left the interstitial space (white arrowhead) which was surrounded by neutrophils and macrophages. Green cells are neutrophils, red cells macrophages and grey cells parasites.

to skin, suggesting that a migration of neutrophils to site of infection might have occurred [141]. Those studies illustrate the importance of neutrophils in direct response towards a parasite but also in regulating the overall fish immune response.

The TLR signalling pathway has been found activated together with neutrophil migration as a first response to *I. multifiliis* in Tibetan highland fish (*Gymnocypris przewalskii*) [169]. TLRs also activate production of inflammatory cytokines in macrophages. Expression of the pro-inflammatory cytokine *il1β* is increased early after infection [142,167,170] and may drive macrophage production of various components such Cox-2 and Mhc II [171]. Cox-2 is suggested to be an enhancer of inflammation in the initial phase of infection [172] whereas Mhc II is linked to adaptive immunity [163]. Additionally, pro-inflammatory cytokines produced by macrophages stimulate the synthesis of macrophage-derived chemokines as well as improving the macrophages phagocytic capacity [173]. An infection with *I. multifiliis* stimulates the production of the chemokine Cxcl8 (Il8) [137,170,174,175], which acts as a chemoattractant for neutrophils to the site of injury. The increase of *cxcl8* followed an increase of *Il1β* [174]. The chemokine receptor *cxcr1* was also found elevated following an *I. multifiliis* infection [142,175] correlating well with the regulation of its ligand, Cxcl8. The interaction between Cxcl8 and Cxcr1 promotes chemotaxis as well as phagocytosis [176]. Other chemokines and receptors such as Cxcl12 and C-C motif chemokine receptor like 1 were found upregulated in the Tibetan highland fish [169], which may further suggest that immune cells are important mediators of protection against this parasite; however, the genes responsible for initiating the response might differ between fish species. *I. multifiliis* infection in channel catfish resulted in an early upregulation of *tlr1* and *tlr9* [158,167,177]. This strongly supports immediate activation of TLRs inducing an immune response of innate origin. TLRs are also involved in the activation of different downstream signalling pathways such as NF-$_{K}$B and MAPK through tumour necrosis factor receptor-associated factor 6 (Traf6) and transforming growth factor-b-activated kinase 1 (Tak1) both of which is expressed by macrophages [178,179]. Studies have shown that Traf6 and Tak1 deficient macrophages cannot activate NF-$_{K}$B and MAPK resulting in a lack of inflammatory cytokines [180]. Both *traf6* and *tak1* have been found upregulated in grass carp following *I. multifiliis* infection [181] indicating the importance of macrophages in the production of cytokines through TLRs.

3.3. Neutrophils and tissue regeneration

3.3.1. Tailfin regeneration

Zebrafish possess a remarkable ability to regenerate several organs, including heart, fin and spinal cord [134,182–184] with evidence building that the innate immune system can influence these regenerative capabilities. Many models now exist in the zebrafish to investigate the regeneration process [185] and especially for studying the roles of the immune cells [186]. In zebrafish larvae, it takes around 4–5 days from tail amputation until complete regeneration [182]. With the use of zebrafish reporter lines with fluorescent immune cells, it is possible to visualise the behaviour of the cells during injury and regeneration [186]. Additionally, as the regenerated tail and the uninjured tail appears similar even after several injuries, regeneration studies can be performed repeatedly on the same animals [187]. Following tissue damage, neutrophils and macrophages are responsible for removal of cell debris and foreign organisms and, through this process, assist in regeneration of damaged tissue [188]. Neutrophils have been shown to be the dominant cell type during the first immediate inflammatory response and are responsible for removal of cell debris and microorganisms [186]. The removal and killing of organisms by neutrophils are facilitated through different anti-microbial properties such as phagocytosis, production of reactive oxygen species and extracellular traps [189]. These anti-microbial reactions are suitable for clearing an infection. However, for regeneration purposes, they can be a challenge both in the short and long term [188]. In the first phase after injury, neutrophils appear to inhibit regeneration. This has been documented by a decreased regeneration time following neutrophil ablation [186] indicating a neutrophil-specific role in tissue regeneration. However, the non-specificity of the anti-microbial properties of neutrophils can be detrimental to the fish tissue itself [190]. When either apoptosis or reverse migration are impaired, neutrophils remain in the regenerating area for an extended period, resulting in a delay in the regeneration [116,188].

4. Zebrafish macrophages

4.1. Macrophage development

Macrophages are a key phagocytic leukocyte population that have a wide variety of roles, from infection clearance to tissue repair and regeneration [115,183,191]. Herbomel and colleagues initially defined zebrafish macrophage development and function by exploiting the transparent nature of embryos using light microscopy methods [192]. Initially identified based on their amoeboid and highly dynamic morphology, larval macrophages were observed to patrol the blood vessels. Importantly, these cells demonstrated phagocytic ability through clearing of apoptotic debris and by responding to bacterial pathogens, behaviours that are homologous to human macrophages [192]. In mammals, macrophages, along with dendritic cells (DCs), are considered key APCs of the innate immune system [193]. Although adult zebrafish are known to have antigen-presenting DCs, it is unclear as to precisely when mature dendritic cells develop in the larval zebrafish [194,195]. Recent transcriptomic work identified a clear distinction between macrophages and DCs in adult zebrafish, with DCs being the cell type enriched in MHC-II antigen-presenting molecules [196]. These data suggest that DCs, rather than macrophages, are the primary APCs in zebrafish. Furthermore, as zebrafish do not develop a functional adaptive immune system until beyond 3 weeks post-fertilisation, the need for DCs to bridge the gap between the innate and adaptive immune system is not required [197], challenging the canonical view that the adaptive immune response is needed to drive macrophage polarisation [26]. Consequently, this dogma poses that the innate immune response is able to initiate polarisation to directly respond to a range of stimuli, using T helper cells to amplify, stabilise and co-ordinate this response in a positive feedback loop [26,198–200].

Since early identification and early descriptions of zebrafish macrophages, the development of transgenic tools to visualise and manipulate macrophages *in vivo* has provided exciting experimental opportunities to uncover new observations about macrophage development and behaviours in health and disease, many of which have since been shown in human systems [78,115,201,202]. Current macrophage zebrafish transgenic reporters include the widely used pan-macrophage *mpeg1.1:mCherry*, *mfap4:tdTomato, fms:mCherry* promoter driven lines, which have been extensively used as tools for macrophage behavioural studies *in vivo* [203–207]. However, used alone, transgenic lines do not conclusively differentiate between macrophages and monocytes, nor tissue-resident and monocyte derived macrophage populations [201,202,208,209]. Despite these challenges, there is emerging evidence that supports the presence of monocytes and monocyte-derived macrophages in zebrafish. Using transgenic lines such as *mpeg1:mCherry*, Moyse and Richardson identified a small population of cells hypothesised to be monocytes in an adult zebrafish heart injury model [210]. In larvae, Ccr2+ putative monocytes, but not tissue-resident macrophages, are recruited during mycobacterial infection [211,212]. However, further work is needed to characterise whether cells that differentially express Ccr2 are true populations of monocytes and macrophages [211–213]. Whilst the current literature likely focusses on the role of tissue-resident macrophages in zebrafish as a consequence of this limitation, there is a growing body of evidence revealing the heterogeneity of these populations both in adults and larvae, some of which will be discussed in

section 4.1.2 [214].

4.1.1. Macrophage ontogeny

In zebrafish, myeloid cell precursors originate from the anterior lateral plate mesoderm, where they migrate to the yolk sac before extravasating and tissue colonisation (Fig. 2) [192,215,216]. This primitive wave occurs as early as 20 hpf, prior to the presence of other leukocytes, such as neutrophils [192]. At 24 hpf, with the onset of blood circulation, the transient definitive wave of haematopoiesis occurs in the PBI, where primitive erythromyeloid progenitors differentiate and primitive macrophages begin to circulate in the blood flow [45,192,215–219]. The PBI transitions into the CHT at 48 hpf, by which point the progenitor population is replaced by HSCs, enabling the second wave of definitive haematopoiesis [45,192,215–219]. During the second wave, HSCs differentiate into macrophage precursors which invade and seed tissues throughout the zebrafish, differentiating into tissue-resident macrophages, including microglia, the specialised macrophages that patrol the central nervous system (CNS) [45,217,220,221]. The pronephric kidney marrow develops from 4 days post fertilization (dpf) and becomes the adult haematopoiesis site from approximately 7 dpf. Migration of HSCs to the developing kidney results in the gradual replacement of the embryonic haematopoietic system [36,215,218]. Comparative descriptions of the origins and timings of the innate immune systems between fish and mammals have been reviewed extensively elsewhere [195,197].

4.1.2. Tissue-resident macrophages

Zebrafish macrophage transgenic reporter lines have been used alongside transcriptomic methods to allow macrophage-specific transcriptomic studies, which have identified distinct tissue-specific macrophage populations in zebrafish, with gene expression signatures found to be conserved with mammals [203–207]. Zebrafish microglia (brain resident macrophages) share significant homology with human microglia. Whilst there are microglia-specific transgenic lines (e.g.: *ApoE:GFP*), most of the literature examining microglia interactions use lines that do not differentiate macrophages and microglia (i.e.: *mpeg:mCherry*) by focusing on the brain [183,222,223]. By isolating *mpeg*+ cells from zebrafish heads, microglia gene expression has been interrogated, identifying many genetic similarities between larval zebrafish microglia and adult human microglia [206]. Furthermore, the heterogeneity seen in human microglia has also been functionally identified in adult zebrafish, emphasising a high degree of conservation between species [207,224]. Other tissue resident macrophage populations are beginning to be identified in zebrafish, with Guilliams et al., recently combining various spatial transcriptomic techniques to identify the transcriptome of hepatic non-parenchymal cells in an unbiased way and Zhou et al., revealing tissue resident macrophage heterogeneity [196,205]. Furthermore, proteogenomic atlases have identified conserved Kupffer cell (liver resident macrophages) profiles between zebrafish and a range of vertebrates, including human (macaques, pig, hamster, chicken, and human) [205]. Most of the studies of tissue resident macrophages are performed on larvae, due to their transparent nature allowing high-content imaging of macrophage populations. Translating transgenic line research into adult zebrafish is challenging due to the loss of transparency and loss of specificity of these genetic markers in adulthood. For example, *mpeg*+ cells in adult zebrafish have recently been identified to also include a subpopulation of B lymphocytes and metaphocytes, limiting the use of this line to examine macrophages beyond larval stages [225,226]. Nevertheless, these datasets highlight how zebrafish have *bona fide* tissue-resident macrophages which behave and have genetic profiles homologous to their human counterparts.

4.2. Macrophage responses to immune challenges

4.2.1. Macrophage polarisation

Macrophage polarisation is a process by which macrophages plastically change their phenotype to adapt to their microenvironment in homeostasis and disease. Macrophage plasticity is conserved in fish, with carp monocytes able to polarise towards M1 (pro-inflammatory) or M2 (wound healing) phenotypes from monocytes (M0) *in vitro*, with pro- and anti-inflammatory marker expression akin to mammals (Fig. 4) [203,227,228]. Due to the large blood volume of larger fish such as carp, they have proven useful models for examining macrophage polarisation in fish, as monocytes can be isolated and cultured to obtain macrophages *in vitro* [228–230]. Markers for M1 and M2 activation states have been identified in macrophages from various teleost fish species, including the common carp, grass carp, ayu and spotted green pufferfish [228–230]. Furthermore, qPCR analyses have found that fish macrophages express M2-associated genes following glucocorticoid exposure (in zebrafish) and infection with the parasite *Lepeophtheirus salmonis* (in Atlantic salmon), like human macrophages [231,232].

One of the advantages of using zebrafish as a model organism to study immune activation is their transparency, as it allows us to examine zebrafish macrophage polarisation using transgenic lines and imaging/transcriptomics analysis [115,203,233]. Isolation and RNAseq analysis of macrophages from transgenic zebrafish (*mpeg1:mCherry*) confirmed that the genetic signature of pro-inflammatory and anti-inflammatory macrophages is broadly conserved with humans [204]. Similar to mammalian macrophages, infection or injury in zebrafish results in macrophage expression of pro-inflammatory markers such as Tnfα, Il1β and inducible nitric oxide synthase (iNos) [115,234,235].

4.2.2. Using zebrafish reporters to delineate macrophage phenotypes in vivo

Pro-inflammatory polarisation of macrophages has become well-characterised in larval zebrafish, and zebrafish models have shed light on their activation *in vivo*. Several zebrafish reporter lines have been generated that have allowed examination of macrophage polarisation over the time course of injury and infectious pathogenesis. To enable real-time visualisation of pro-inflammatory macrophages, promoter driven Tnfα transgenic lines have been generated and are now well-characterised reporters that robustly label pro-inflammatory macrophages [203,242]. Using *tnfα:GFP* lines to examine macrophage dynamics *in vivo*, it was found that pro-inflammatory macrophages (Tnfα+) arrive at wound and infection sites, later switching phenotype to a potential anti-inflammatory phenotype characterised by changes in macrophage shape (amoeboid to elongated) and behaviour (direct to reverse/circular) [191,203,243]. Additional pro-inflammatory transgenic lines which have been used to visualise and characterise pro-inflammatory macrophages in zebrafish include *il-1β:GFP* [115]. Macrophage pro-inflammatory nitric oxide (NO) balance is governed by two enzymes, iNos and arginase (that both use L-arginine as a substrate), and they are often used as dichotomous markers for mammalian M1 and M2 phenotypes respectively *in vitro* and were found to mark carp M1 and M2 populations in a similar way [244]. In zebrafish, the activity of iNos has been measured using an antibody against tyrosine nitrosylation as an indirect measure of macrophage NO [235,245]. As these experiments rely on fixed larvae, the spatial and temporal dynamics of polarised macrophages is lost. Observing anti-inflammatory macrophages in zebrafish *in vivo* is in its infancy, as characterising the anti-inflammatory response has, until recently, relied on analysis of macrophage morphology and extrapolation from data obtained from these pro-inflammatory reporter lines (e.g.: *tnfα*-expressing macrophages vs. *tnfα*-negative macrophages) [191,203,243]. Recently, a zebrafish transgenic anti-inflammatory reporter (*arg2:GFP*) has been developed, allowing for *in vivo* characterisation of the anti-inflammatory response in real time post immune challenge [233]. A small population of macrophages upregulated *arg2:GFP* at early and later stages post bacterial infection, indicative of anti-inflammatory macrophages during infectious pathogenesis [233].

Although the current transgenic tools available are enabling pro-inflammatory macrophages to be extensively characterised, there remains a gap in our understanding of the macrophage anti-inflammatory

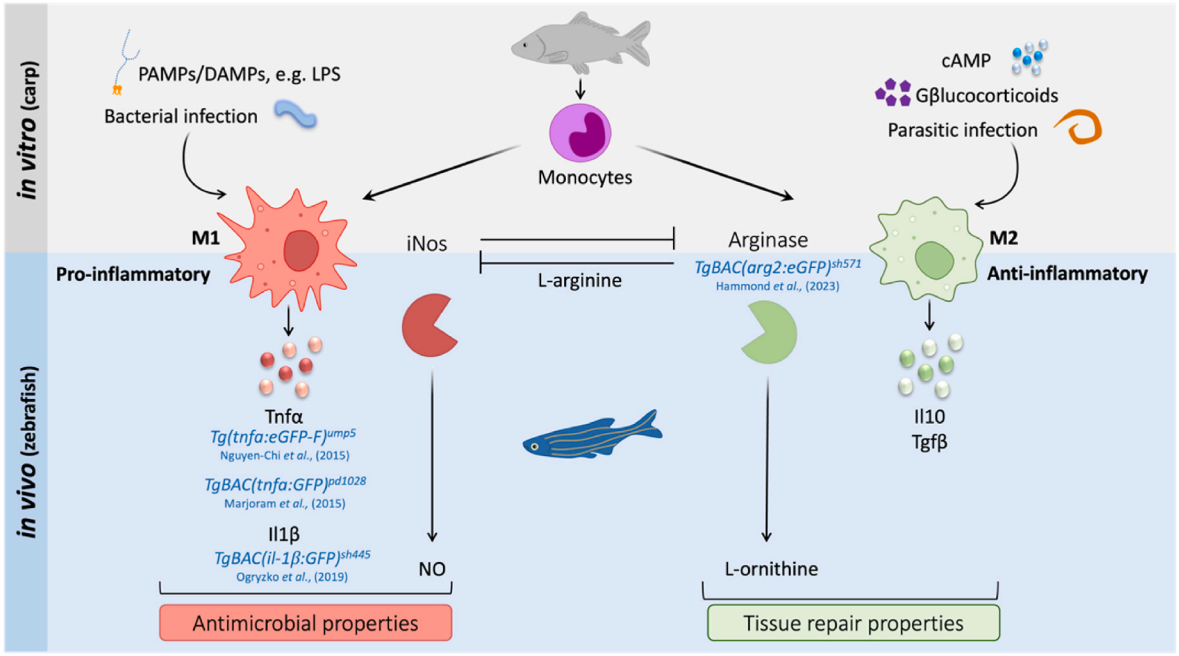

Fig. 4. Macrophages polarise on a continuum in response to different stimuli. Macrophages polarise towards an innate-activated pro-inflammatory phenotype following a range of stimuli, including bacterial pathogens (e.g. *Mycobacterium marinum*) and PAMPs/DAMPs such as LPS [234,236]. Pro-inflammatory macrophages express and release pro-inflammatory cytokines such as tumour necrosis factor-α (Tnfα) and interleukin 1-β (Il1β) [115,234]. A hallmark of pro-inflammatory macrophages is the enzyme iNos, which uses L-arginine as a substrate to produce antimicrobial NO [237]. The antimicrobial immune response of pro-inflammatory macrophages is essential for increased phagocytic activity and consequent pathogen killing. Alternatively, stimuli such as parasitic infection (e.g. *Lepeophtheirus salmonis*) and glucocorticoids can induce anti-inflammatory macrophages [231,232,236,238]. They express and release anti-inflammatory cytokines such as Il10 and transforming growth factor β (Tgfβ) [203,239]. The enzyme arginase is an anti-inflammatory macrophage marker, also using L-arginine as a substrate to produce 'healing' factors such as L-ornithine and polyamines [240]. These anti-inflammatory properties enable this macrophage activation state to promote tissue repair and return of homeostasis. References for zebrafish transgenic lines currently in use included within the figure. Abbreviations: PAMPs/DAMPs, pattern-/-damage-associated molecular patterns; LPS, lipopolysaccharides; iNos, inducible nitric oxide synthase; NO, nitric oxide. Adapted from Refs. [26,241].

response. Transgenic lines such as *tnfa:GFP* and *arg2:GFP* will allow us to get a more complete understanding of the plasticity of macrophages to polarise (and re-polarise) in response to microenvironmental cues. The interplay of pro- and anti-inflammatory macrophages in zebrafish is a growing field, and with most of the studies mentioned above looking at larval zebrafish prior to the development of the adaptive immune system, zebrafish research is adding to the emerging dogma which challenges the traditional view that adaptive immune T_H1 and T_H2 responses are required to drive macrophage polarisation [26].

4.2.3. *Macrophage function in infection and injury*

Macrophages rapidly respond to immune challenges such as infection and injury, playing key roles in host defence [246,247]. The activation status of macrophages dictates how well zebrafish can respond to these immune challenges and recover and restore tissue homeostasis once the challenge has been cleared. An initial transient pro-inflammatory response is required for phagocytosis of pathogens and tissue debris, switching to anti-inflammatory, wound healing phenotypes at later stages to promote timely resolution of inflammation and tissue repair [191,203,231,234,243,248,249]. Two immune challenges have given important insights into the role of macrophages in these clinically important processes: *Mycobacterium marinum* (bacterial infection) and tail fin transection (injury) models.

4.2.3.1. Mycobacterium infection. Zebrafish larvae infected with *Mycobacterium marinum* have been successfully used as an *in vivo* tuberculosis model for the last 20 years, with many important observations made on macrophage and mycobacterial interaction [115,211,234,249–253]. Infection with *M. marinum* results in the formation of hallmark, macrophage-driven, aggregates of immune cells around infection (granulomas). The bacterium can survive, replicate and disseminate in zebrafish macrophages/granulomas, just as in human tuberculosis. Macrophage phagocytosis and activation is essential for proper control of *M. marinum*. Genetic ablation of macrophages by *pu.1* morpholino knockdown greatly increases the bacterial burden of the larvae, though *M. marinum* failed to disseminate to additional tissues in the absence of macrophages, indicating a role for macrophages in bacterial dissemination [254]. The migratory ability of macrophages, such as that governed by the Cxcr3-Cxcl11 chemokine axis, also plays a key role in *M. marinum* dissemination. *cxcr3.2* mutant zebrafish had increased macrophage motility and *M. marinum* dissemination but were also more microbicidal due to enlarged lysosomes [255,256]. Knockdown of Marco, a macrophage scavenger receptor, not only reduced *M. marinum* phagocytosis, but also reduced the pro-inflammatory cytokine profile (e.g. *il1β*), leading to an increase in bacterial burden, indicating a need for macrophage control of infection [249]. Using the pro-inflammatory transgenic lines mentioned previously, macrophages were shown to transcriptionally upregulate *il1β:GFP* and *tnfα:GFP* early in *M. marinum* infection pathogenesis [115,234]. Furthermore, genetic stabilisation of Hif-1α, a pro-inflammatory transcription factor, not only upregulated expression of these pro-inflammatory signals in macrophages, but also improved *M. marinum* clearance [115,234]. Signalling pathways which underpin macrophage polarisation, such as induction of *tnfα:GFP* via a cyclooxygenase/prostaglandin E2 axis, have also been uncovered by using *tnfα* promoter driven lines as *in vivo* readouts [234]. However, an appropriate pro-inflammatory response is important for infection control. Excessive Tnfα can also result in bacterial dissemination, as it has been associated with necrosis of *M. marinum* -infected macrophages caused by mitochondrial calcium overload [257]. At later stages of infection, *M. marinum* exploits macrophages by polarising them towards a more anti-inflammatory phenotype and preferentially infecting M2-like/anti-inflammatory macrophages as a survival strategy [252].

M. marinum can use cell surface lipids to act as a physical barrier to prevent TLR-mediated pro-inflammatory macrophage detection [211]. Instead, *M. marinum* phenolic glycolipids (PGL) allows for infection of anti-inflammatory macrophages, followed by induction of Ccl2 chemokine production to recruit monocytes to facilitate its escape, creating a protective niche that enables bacterial expansion [211,212,247]. Macrophage-expressed gene 1 (*mpeg1*) is a gene encoding perforin-2, a pore-forming protein that is a key player in macrophage bactericidal activity. *mpeg1* expressing macrophages take part in granuloma formation, but *M. marinum* then downregulate the *mpeg1* promoter, possibly to dampen macrophage antimicrobial function [250]. Macrophages within zebrafish granulomas were found to robustly express E-cadherins, demonstrating *M. marinum* -mediated macrophage-to-epithelioid reprogramming, making them inherently less pro-inflammatory [251,252]. Together, these studies add to the growing body of evidence that *M. marinum* can transcriptionally shift macrophages away from proinflammatory phenotypes towards anti-inflammatory, permissive, phenotypes.

4.2.3.2. Tail fin transection (injury). To investigate the macrophage response to sterile injury, in the absence of infection, a zebrafish tail fin transection (amputation) model has been widely adopted. To track macrophage recruitment to the tail fin injury site, the *mpeg1:Kaede* line has been adopted, whereby macrophages express the Kaede protein, which photoconverts from green to red fluorescence with UV light. By photoconverting macrophages at the injury site to red, it can then be determined whether a separate, potentially anti-inflammatory, macrophage population arrives at later stages of regeneration. This technique has allowed us to visualise for the first time *in vivo*, that macrophages are recruited to the injury site in a single wave, changing phenotype throughout the healing and regeneration process [243]. By using *tnfa:GFP* transgenic larvae it has been demonstrated in a number of studies that macrophages are rapidly recruited to the injury site where they accumulate, peaking at 6 h post-amputation (hpa), expressing *tnfα* early after injury and downregulating expression at later stages [191,203,243,258,259]. The initial macrophage pro-inflammatory response seen early after tail fin transection is vital for wound healing and the regenerative success of the tail fin tissue [191]. Interestingly, though *tnfα*-expressing macrophages were observed at the early stages of recruitment and accumulation at the injury site, RT-qPCR analysis of inflammatory genes, such as *tnfα* and *il6*, found that mRNA expression levels at a tissue level remained largely unchanged throughout the regenerative process, though this does not interrogate macrophage expression directly [260]. Using FACs purification, Nguyen-Chi et al., isolated *tnfα*+ and *tnfα*-negative macrophages, identifying distinct pro- and anti-inflammatory molecular signatures, respectively [203,243].

Evidence for an anti-inflammatory/M2 switch at later stages of tail fin injury is currently limited to indirect observations, but more direct evidence has recently been emerging. Flow cytometry of *tnfα:GFP* larvae confirmed that at later stages of inflammation, only 5.6% of macrophages remained *tnfα*+, suggesting a switch towards an M2-like phenotype [203]. These observations correlated with a shift from a pro-inflammatory (*tnfα, il1β, il6*) to an anti-inflammatory (*tgfβ1, ccr2*) transcriptional profile identified using qRT-PCR of *tnfα*+ and *tnfα*-macrophages [203,243]. Unbiased transcriptomic analysis suggest that macrophages independently transition through a linear sequence of anti-inflammatory activation states [248]. Recently, an *arg2:GFP* expressing macrophage subpopulation was observed at the tail fin during regenerative stages, the first direct evidence of anti-inflammatory macrophages using an anti-inflammatory transgenic line in zebrafish [233].

4.3. Macrophages and tissue regeneration

4.3.1. Tail fin regeneration

As mentioned in section 3.3, zebrafish are able to functionally regenerate various tissues after injury, in many cases without the formation of a scar [183]. One of the mechanisms for regenerative success may be the biphasic innate immune response to injury, characterised by a transient pro-inflammatory response, which is then replaced by a pro-longed anti-inflammatory response [261,262]. Macrophages play an essential role in the regenerative process [183,191]. Larvae that are depleted of macrophages (e.g. *irf8*$^{-/-}$) have greatly impaired regenerative capacity [191,262]. Whilst one could be forgiven for thinking that anti-inflammatory macrophages are responsible for regeneration, there is a growing body of evidence demonstrating the importance of pro-inflammatory macrophages for successful regeneration [191,203,243,262,263].

As the larval zebrafish tail fin can regenerate within 3 days, tail fin transection is a widely studied model of zebrafish regeneration, giving new insights into the *in vivo* role of macrophages in this process. Impaired tail fin regeneration was observed following chemical and genetic depletion of macrophages at different time-points to target pro-inflammatory or anti-inflammatory macrophages, demonstrating the importance of both phenotypes in this process [191]. Following the pro-inflammatory stage, *tnfα* negative macrophages accumulate up until 3 days post-amputation, suggesting that anti-inflammatory macrophages are present during the later stages of the regenerative process [191]. Tnfα secreted from pro-inflammatory macrophages has proven to be pivotal for tail fin regenerative success, demonstrating the importance of pro-inflammatory macrophages for the regenerative process [191]. Macrophage phenotype must switch away from a pro-inflammatory phenotype at later stages to ensure regenerative success, with excessive pro-inflammatory macrophage signalling resulting in impaired regeneration and collateral tissue damage [184]. A study using mutant zebrafish larvae that have a reduced pool of peripheral tissue-resident macrophages (*csf1ra*$^{-/-}$) suggests that this macrophage population enables efficient tail fin regeneration by dampening the pro-inflammatory environment [214]. Macrophage polarisation can be therapeutically targeted to modulate tissue regeneration. Treatment with Protectin D1, a pro-resolving mediator, accelerated changes in macrophage behaviour, correlating with increased expression of anti-inflammatory markers (e.g. *tgf1*, *ccr2*) identified by RT-qPCR, leading to accelerated tail fin regeneration [184]. All studies to date use *tnfα*-driven transgenic lines to visualise pro-inflammatory macrophages *in vivo*, identifying potential anti-inflammatory macrophage subpopulations through extrapolation of this data (i.e.: *tnfα* negative macrophages) [183,191,262]. Novel transgenics such as the *arg2:GFP* line could allow investigation of both pro- and anti-inflammatory macrophage roles in the finely-tuned responses that allow zebrafish to have this regenerative capacity [233].

5. Conclusion

It is clear that fish have an important role to play in driving our understanding of the immune response to external challenges. Zebrafish have proven their usefulness as models of inflammation and infection through a multitude of studies, uncovering novel mechanisms through assays involving *in vivo* imaging, genetic manipulation and drug screening. Exploiting these key advantages of the zebrafish have uncovered the plasticity of fish macrophages and neutrophils, and how they are able to alter their activation status to respond to different immune challenges. The development of transgenic and mutant zebrafish lines provides us with the tools to examine leukocyte pro- and anti-inflammatory signalling in these contexts, allowing us to further understand the dynamic role of these critical innate immune cell populations *in vivo*. Furthermore, the increasing body of evidence demonstrating functional conservation between zebrafish and

mammalian leukocytes, highlights the value of studying zebrafish macrophage and neutrophil biology to understand human disease. There is, however, a lack of studies on the collaboration and interactions of neutrophils and macrophages, and this should be prioritised in the future, as fine-tuning both cell-specific responses could provide more precise therapeutic intervention for human disease.

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CRediT authorship contribution statement

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Data availability

No data was used for the research described in the article.

References

[1] C. Heip, H. Hummel, P. van Avasaath, W. Appeltans, C. Arvanitidis, R. Aspden, et al., Marine Biodiversity and Ecosystem Functioning, 2009. Printbase, Dublin, Ireland.
[2] P. Aleström, L. D'Angelo, P.J. Midtlyng, D.F. Schorderet, S. Schulte-Merker, F. Sohm, et al., Zebrafish: Housing and husbandry recommendations, Lab. Anim 54 (2020) 213–224, https://doi.org/10.1177/0023677219869037.
[3] N.S. Trede, D.M. Langenau, D. Traver, A.T. Look, L.I. Zon, The use of zebrafish to understand immunity, Immunity 20 (2004) 367–379, https://doi.org/10.1016/S1074-7613(04)00084-6.
[4] S.R. Paludan, T. Pradeu, S.L. Masters, T.H. Mogensen, Constitutive immune mechanisms: mediators of host defence and immune regulation, Nat. Rev. Immunol. 21 (2021) 137–150, https://doi.org/10.1038/s41577-020-0391-5.
[5] C. Uribe, H. Folch, R. Enriquez, G. Moran, Innate and adaptive immunity in teleost fish: a review, Vet Med-Czech 56 (2011) 486–503, https://doi.org/10.17221/3294-VETMED.
[6] K. Buchmann, Evolution of innate immunity: clues from invertebrates via fish to mammals, Front. Immunol. 5 (2014), https://doi.org/10.3389/fimmu.2014.00459.
[7] T. Oosting, B. Star, J.H. Barrett, M. Wellenreuther, P.A. Ritchie, N.J. Rawlence, Unlocking the potential of ancient fish DNA in the genomic era, Evol Appl 12 (2019) 1513–1522, https://doi.org/10.1111/eva.12811.
[8] D. Davesne, M. Friedman, A.D. Schmitt, V. Fernandez, G. Carnevale, P.E. Ahlberg, et al., Fossilized cell structures identify an ancient origin for the teleost whole-genome duplication, Proc. Natl. Acad. Sci. U.S.A. 118 (2021), https://doi.org/10.1073/pnas.2101780118.
[9] D.M. Mokhtar, G. Zaccone, A. Alesci, M. Kuciel, M.T. Hussein, R.K.A. Sayed, Main components of fish immunity: an overview of the fish immune system, Fishes 8 (2023), https://doi.org/10.3390/fishes8020093.
[10] G.F. Wiegertjes, P.M. Elks, Fish macrophages, in: K. Buchmann, C.J. Secombes (Eds.), Principles of Fish Immunology, Springer, Switzerland, 2022, pp. 203–228, https://doi.org/10.1007/978-3-030-85420-1_6.
[11] C.J. Thomas, K. Schroder, Pattern recognition receptor function in neutrophils, Trends Immunol. 34 (2013) 317–328, https://doi.org/10.1016/j.it.2013.02.008.
[12] Y. Li, Y. Li, X. Cao, X. Jin, T. Jin, Pattern recognition receptors in zebrafish provide functional and evolutionary insight into innate immune signaling pathways, Cell. Mol. Immunol. 14 (2017) 80–89, https://doi.org/10.1038/cmi.2016.50.
[13] M. Varga, The Doctor of delayed publications: the remarkable life of George Streisinger (1927-1984), Zebrafish 15 (3) (2018) 314–319, https://doi.org/10.1089/zeb.2017.1531.
[14] S.A. Renshaw, C.A. Loynes, D.M. Trushell, S. Elworthy, P.W. Ingham, M.K. B. Whyte, A transgenic zebrafish model of neutrophilic inflammation, Blood 108 (2006) 3976–3978, https://doi.org/10.1182/blood-2006-05-024075.
[15] F. Ellett, L. Pase, J.W. Hayman, A. Andrianopoulos, G.J. Lieschke, mpeg1 promoter transgenes direct macrophage-lineage expression in zebrafish, Blood 117 (2011) E49–E56, https://doi.org/10.1182/blood-2010-10-314120.
[16] H. Bjørgen, E.O. Koppang, Anatomy of teleost fish immune structures and organs, in: K. Buchmann, C. Secombes (Eds.), Principles of Fish Immunology, Springer, Cham, Switzerland, 2022, pp. 1–30, https://doi.org/10.1007/s00251-020-01196-0.
[17] G. Scapigliati, A. Miccoli, F. Buonocore, A.M. Fausto, S. Picchietti, Lymphocytes of teleosts, in: K. Buchmann, C.J. Secombes (Eds.), Principles of Fish Immunology, Springer, Switzerland, 2022, pp. 177–2022, https://doi.org/10.1007/978-3-030-85420-1_5.
[18] B. Star, S. Jentoft, Why does the immune system of Atlantic cod lack MHC II? Bioessays 34 (2012) 648–651, https://doi.org/10.1002/bies.201200005.
[19] S.A. Bjørnestad, M.H. Solbakken, K.S. Jakobsen, S. Jentoft, O. Bakke, C. Progida, Atlantic cod (*Gadus morhua*) MHC I localizes to endolysomal compartments independently of cytosolic sorting signals, Front. Cell Dev. Biol. 11 (2023), https://doi.org/10.3389/fcell.2023.1050323.
[20] B. Magnadóttir, Innate immunity of fish (overview), Fish Shellfish Immunol. 20 (2006) 137–151, https://doi.org/10.1016/j.fsi.2004.09.006.
[21] R.A. Dalmo, J. Bøgwald, Innate immunity, in: K. Buchmann, C. Secombes (Eds.), Principles of Fish Immunology, Springer, Cham, Switzerland, 2022, pp. 31–104, https://doi.org/10.1007/978-3-030-85420-1_2.
[22] C. Hohn, L. Petrie-Hanson, Rag1-/- mutant zebrafish demonstrate specific protection following bacterial re-exposure, PLoS One 7 (2012), https://doi.org/10.1371/journal.pone.0044451.
[23] G. Wigmore, Cod genome reveals unusual immune system, Nature (2011), https://doi.org/10.1038/news.2011.470.
[24] L. Pilström, G.W. Warr, S. Strömberg, Why is the antibody response of Atlantic cod so poor? The search for a genetic explanation, Fish. Sci. 71 (2005) 961–971, https://doi.org/10.1111/j.1444-2906.2005.01052.x.
[25] J.J. Havixbeck, D.R. Barreda, Neutrophil development, migration, and function in teleost fish, Biology 4 (2015) 715–734, https://doi.org/10.3390/biology4040715.
[26] G.F. Wiegertjes, A.S. Wentzel, H.P. Spaink, P.M. Elks, I.R. Fink, Polarization of immune responses in fish: the 'macrophages first' point of view, Mol. Immunol. 69 (2016) 146–156, https://doi.org/10.1016/j.molimm.2015.09.026.
[27] J. Resseguier, M. Nguyen-Chi, J. Wohlmann, D. Rigaudeau, I. Salinas, S. H. Oehlers, et al., Identification of a pharyngeal mucosal lymphoid organ in zebrafish and other teleosts: tonsils in fish? Sci. Adv. 9 (2023) https://doi.org/10.1126/sciadv.adj0101.
[28] A.M. Earley, C.L. Graves, C.E. Shiau, Critical role for a subset of intestinal macrophages in shaping gut microbiota in adult zebrafish, Cell Rep. 25 (2018) 424–436, https://doi.org/10.1016/j.celrep.2018.09.025.
[29] P.W. Kania, K. Buchmann, Complement activation in fish with emphasis on MBL/MASP, in: K. Buchmann, C.J. Secombes (Eds.), Principles of Fish Immunology, Springer, Switzerland, 2022, pp. 279–300, https://doi.org/10.1007/978-3-030-85420-1_9.
[30] H. Boshra, J. Li, J.O. Sunyer, Recent advances on the complement system of teleost fish, Fish Shellfish Immunol. 20 (2006) 239–262, https://doi.org/10.1016/j.fsi.2005.04.004.
[31] M.C. Holland, J.D. Lambris, The complement system in teleosts, Fish Shellfish Immunol. 12 (2002) 399–420, https://doi.org/10.1006/fsim.2001.0408.
[32] A. Schreiber, H. Xiao, J.C. Jennette, W. Schneider, F.C. Luft, R. Kettritz, C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis, J. Am. Soc. Nephrol. 20 (2009) 289–298, https://doi.org/10.1681/ASN.2008050497.
[33] H. Bai, L. Mu, L. Qiu, N. Chen, J. Li, Q. Zeng, et al., Complement C3 regulates inflammatory response and monocyte/macrophage phagocytosis of *Streptococcus agalactiae* in a teleost fish, Int. J. Mol. Sci. 23 (2022), https://doi.org/10.3390/ijms232415586.
[34] H. Matsuyama, T. Yano, T. Yamakawa, M. Nakao, Opsonic effect of the third complement component (C3) of carp (*Cyprus carpio*) on phagocytosis by neutrophils, Fish Shellfish Immunol. 2 (1992) 69–78, https://doi.org/10.1016/S1050-4648(06)80028-8.
[35] C. Summers, S.M. Rankin, A.M. Condliffe, N. Singh, A.M. Peters, E.R. Chilvers, Neutrophil kinetics in health and disease, Trends Immunol. 31 (2010) 318–324, https://doi.org/10.1016/j.it.2010.05.006.
[36] M. Jagannathan-Bogdan, L.I. Zon, Hematopoiesis, Development 140 (2013) 2463–2467, https://doi.org/10.1242/dev.083147.
[37] A.V. Gore, L.M. Pillay, M.V. Galanternik, B.M. Weinstein, The zebrafish: a fintastic model for hematopoietic development and disease, Wiley Interdiscip Rev Dev Biol 7 (2018), https://doi.org/10.1002/wdev.312.
[38] D. Traver, P. Herbomel, E.E. Patton, R.D. Murphey, J.A. Yoder, G.W. Litman, et al., The zebrafish as a model organism to study development of the immune system, Adv. Immunol. 81 (2003) 254–330, https://doi.org/10.1016/S0065-2776(03)81007-6.
[39] I. Kobayashi, S. Kuniyoshi, K. Saito, T. Moritomo, T. Takahashi, T. Nakanishi, Long-term hematopoietic reconstitution by transplantation of kidney hematopoietic stem cells in lethally irradiated clonal ginbuna crucian carp (*Carassius auratus langsdorfii*), Dev. Comp. Immunol. 32 (2008) 957–965, https://doi.org/10.1016/j.dci.2008.01.006.
[40] H.W. Detrich, M.W. Kieran, F.Y. Chan, L.M. Barone, K. Yee, J.A. Rundstadler, et al., Intraembryonic hematopoietic cell migration during vertebrate development,

Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 10713–10717, https://doi.org/10.1073/pnas.92.23.10713.
[41] M.A. Thompson, D.G. Ransom, S.J. Pratt, H. MacLennan, M.W. Kieran, H. W. Detrich, et al., The cloche and spadetail genes differentially affect hematopoiesis and vasculogenesis, Dev. Biol. 197 (2) (1998) 248–269, https://doi.org/10.1006/dbio.1998.8887.
[42] J.L.O. de Jong, L.I. Zon, Use of the zebrafish system to study primitive and definitive hematopoiesis, Annu. Rev. Genet. 39 (2005) 481–501, https://doi.org/10.1146/annurev.genet.39.073003.095931.
[43] C.E. Willett, A. Cortes, A. Zuasti, A.G. Zapata, Early hematopoiesis and developing lymphoid organs in the zebrafish, Dev. Dynam. 214 (1999) 323–336, https://doi.org/10.1002/(SICI)1097-0177(199904)214:4<323::AID-AJA5>3.0.CO;2-3.
[44] D. Le Guyader, M.J. Redd, E. Colucci-Guyon, E. Murayama, K. Kissa, V. Briolat, et al., Origins and unconventional behavior of neutrophils in developing zebrafish, Blood 111 (2008) 132–141, https://doi.org/10.1182/blood-2007-06-095398.
[45] J.Y. Bertrand, A.D. Kim, E.P. Violette, D.L. Stachura, J.L. Cisson, D. Traver, Definitive hematopoiesis initiates through a committed erythromyeloid progenitor in the zebrafish embryo, Development 134 (2007) 4147–4156, https://doi.org/10.1242/dev.012385.
[46] H. Jin, R. Sood, J. Xu, F. Zhen, M.A. English, P.P. Liu, et al., Definitive hematopoietic stem/progenitor cells manifest distinct differentiation output in the zebrafish VDA and PBI, Development 136 (2009) 647–654, https://doi.org/10.1242/dev.029637.
[47] J.B. Cowland, N. Borregaard, Granulopoiesis and granules of human neutrophils, Immunol. Rev. 273 (2016) 11–28, https://doi.org/10.1111/imr.12440.
[48] P. Lacy, Mechanisms of degranulation in neutrophils, All Asth Clin Immun 2 (3) (2006) 98–108, https://doi.org/10.1186/1710-1492-2-3-98.
[49] N. Borregaard, J.B. Cowland, Granules of the human neutrophilic polymorphonuclear leukocyte, Blood 89 (1997) 3503–3521, https://doi.org/10.1182/blood.V89.10.3503.
[50] D.F. Bainton, J.L. Ullyot, M.G. Farquhar, The development of neutrophilic polymorphonuclear leukocytes in human bone marrow, J. Exp. Med. 134 (4) (1971) 907–934, https://doi.org/10.1084/jem.134.4.907.
[51] S. von Vietinghoff, K. Ley, Homeostatic regulation of blood neutrophil counts, J. Immunol. 181 (2008) 5183–5188, https://doi.org/10.4049/jimmunol.181.8.5183.
[52] C.M. Bennett, J.P. Kanki, J. Rhodes, T.X. Liu, B.H. Paw, M.W. Kieran, et al., Myelopoiesis in the zebrafish, *Danio rerio*, Blood 98 (2001) 643–651, https://doi.org/10.1182/blood.v98.3.643.
[53] K.M. Banks, Y. Lan, T. Evans, Tet proteins regulate neutrophil granulation in zebrafish through demethylation of socs3b mRNA, Cell Rep. 34 (2021), https://doi.org/10.1016/j.celrep.2020.108632.
[54] H.B. Fan, Y.J. Liu, L. Wang, T.T. Du, M. Dong, L. Gao, et al., miR-142-3p acts as an essential modulator of neutrophil development in zebrafish, Blood 124 (2014) 1320–1330, https://doi.org/10.1182/blood-2013-12-545012.
[55] G.J. Lieschke, A.C. Oates, M.O. Crowhurst, A.C. Ward, J.E. Layton, Morphologic and functional characterization of granulocytes and macrophages in embryonic and adult zebrafish, Blood 98 (2001) 3087–3096, https://doi.org/10.1182/blood.v98.10.3087.
[56] M. Wu, Y. Xu, J. Li, J. Lian, Q. Chen, P. Meng, et al., Genetic and epigenetic orchestration of Gfi1aa-Lsd1-cebpa in zebrafish neutrophil development, Development 148 (2021), https://doi.org/10.1242/dev.199516.
[57] H.R. Manley, M.C. Keightley, G.J. Lieschke, The neutrophil nucleus: an important influence on neutrophil migration and function, Front. Immunol. 9 (2018), https://doi.org/10.3389/fimmu.2018.02867.
[58] G. Rey Vázquez, G.A. Guerrero, Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes), Tissue Cell 39 (2007) 151–160, https://doi.org/10.1016/j.tice.2007.02.004.
[59] Y. Sasaki, M. Maita, N. Okamoto, Rainbow trout neutrophils are responsible for non-specific cytotoxicity, Fish Shellfish Immunol. 12 (2002) 243–252, https://doi.org/10.1006/fsim.2001.0368.
[60] S.H. Hamdani, D.N. McMillan, E.F. Pettersen, H. Wergeland, C. Endresen, A. E. Ellis, et al., Isolation of rainbow trout neutrophils with an anti-granulocyte monoclonal antibody, Vet. Immunol. Immunopathol. 63 (1998) 369–380, https://doi.org/10.1016/s0165-2427(98)00115-9.
[61] E.A. Harvie, J.M. Green, M.N. Neely, A. Huttenlocher, Innate immune response to *Streptococcus iniae* infection in zebrafish larvae, Infect. Immun. 81 (2013) 110–121, https://doi.org/10.1128/IAI.00642-12.
[62] B.A. Katzenback, M. Belosevic, Isolation and functional characterization of neutrophil-like cells, from goldfish (*Carassius auratus* L.) kidney, Dev. Comp. Immunol. 33 (2009) 601–611, https://doi.org/10.1016/j.dci.2008.10.011.
[63] J.P. Scharsack, D. Steinhagen, C. Kleczka, J.O. Schmidt, W. Körting, R.D. Michael, et al., Head kidney neutrophils of carp (*Cyprinus carpio* L.) are functionally modulated by the haemoflagellate *Trypanoplasma borreli*, Fish Shellfish Immunol. 14 (2003) 389–403, https://doi.org/10.1006/fsim.2002.0447.
[64] P.T.K. Woo, J.F. Leatherland, D.W. Bruno, Fish Diseases and Disorders, UK CABI International, Wallingford, 2011. ISBN : 978-0-85199-015-6.
[65] G. Dixon, P.M. Elks, C.A. Loynes, M.K. Whyte, S.A. Renshaw, A method for the in vivo measurement of zebrafish tissue neutrophil lifespan, ISRN Hematol 2012 (2012), https://doi.org/10.5402/2012/915868.
[66] E. Kolaczkowska, P. Kubes, Neutrophil recruitment and function in health and inflammation, Nat. Rev. Immunol. 13 (2013) 159–175, https://doi.org/10.1038/nri3399.
[67] C. Rosales, Neutrophil: a cell with many roles in inflammation or several cell types? Front. Physiol. 9 (2018) https://doi.org/10.3389/fphys.2018.00113.
[68] J. Pillay, I. den Braber, N. Vrisekoop, L.M. Kwast, R.J. de Boer, J.A. Borghans, et al., In vivo labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days, Blood 116 (2010) 625–627, https://doi.org/10.1182/blood-2010-01-259028.
[69] M.A. Arnaout, L.L. Lanier, D.V. Faller, Relative contribution of the leukocyte molecules Mo1, LFA-1, and p150,95 (LeuM5) in adhesion of granulocytes and monocytes to vascular endothelium is tissue- and stimulus-specific, J. Cell. Physiol. 137 (1988) 305–309, https://doi.org/10.1002/jcp.1041370214.
[70] J.I. Gallin, M.P. Fletcher, B.E. Seligmann, S. Hoffstein, K. Cehrs, N. Mounessa, Human neutrophil-specific granule deficiency: a model to assess the role of neutrophil-specific granules in the evolution of the inflammatory response, Blood 59 (1982) 1317–1329, https://doi.org/10.1182/blood.V59.6.1317.1317.
[71] T.N. Mayadas, X. Cullere, C.A. Lowell, The multifaceted functions of neutrophils, Annu. Rev. Pathol. 9 (2014) 181–218, https://doi.org/10.1146/annurev-pathol-020712-164023.
[72] M.A. Arnaout, Structure and function of the leukocyte adhesion molecules CD11/CD18, Blood 75 (1990) 1037–1050, https://doi.org/10.1182/blood.V75.5.1037.1037.
[73] P. Niethammer, C. Grabher, A.T. Look, T.J. Mitchison, A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish, Epub 20090603, Nature 459 (2009) 996–999, https://doi.org/10.1038/nature08119.
[74] S.K. Yoo, T.W. Starnes, Q. Deng, A. Huttenlocher, Lyn is a redox sensor that mediates leukocyte wound attraction in vivo, Nature 480 (2011) 109–112, https://doi.org/10.1038/nature10632.
[75] S. de Oliveira, E.E. Rosowski, A. Huttenlocher, Neutrophil migration in infection and wound repair: going forward in reverse, Nat. Rev. Immunol. 16 (2016) 378–391, https://doi.org/10.1038/nri.2016.49.
[76] F.V.S. Castanheira, P. Kubes, Neutrophils and NETs in modulating acute and chronic inflammation, Blood 133 (2019) 2178–2185, https://doi.org/10.1182/blood-2018-11-844530.
[77] Q. Deng, M. Sarris, D.A. Bennin, J.M. Green, P. Herbomel, A. Huttenlocher, Localized bacterial infection induces systemic activation of neutrophils through Cxcr2 signaling in zebrafish, J. Leukoc. Biol. 93 (2013) 761–769, https://doi.org/10.1189/jlb.1012534.
[78] C. Gray, C.A. Loynes, M.K.B. Whyte, D.C. Crossman, S.A. Renshaw, T.J.A. Chico, Simultaneous intravital imaging of macrophage and neutrophil behaviour during inflammation using a novel transgenic zebrafish, Thromb. Haemostasis 105 (2011) 811–819, https://doi.org/10.1160/TH10-08-0525.
[79] H.M. Isles, C.A. Loynes, S. Alasmari, F.C. Kon, K.M. Henry, A. Kadochnikova, et al., Pioneer neutrophils release chromatin within in vivo swarms, Elife 10 (2021), https://doi.org/10.7554/eLife.68755.
[80] H. Poplimont, A. Georgantzoglou, M. Boulch, H.A. Walker, C. Coombs, F. Papaleonidopoulou, et al., Neutrophil swarming in damaged tissue is orchestrated by connexins and cooperative calcium alarm signals, e7, Curr. Biol. 30 (2020) 2761–2776, https://doi.org/10.1016/j.cub.2020.05.030.
[81] T. Lämmermann, P.V. Afonso, B.R. Angermann, J.M. Wang, W. Kastenmüller, C. A. Parent, et al., Neutrophil swarms require LTB_4 and integrins at sites of cell death in vivo, Nature 498 (2013) 371–375, https://doi.org/10.1038/nature12175.
[82] M. Mihlan, K.M. Glaser, M.W. Epple, T. Lämmermann, Neutrophils: amoeboid migration and swarming dynamics in tissues, Front. Cell Dev. Biol. 10 (2022), https://doi.org/10.3389/fcell.2022.871789.
[83] M.K. Brannon, J.M. Davis, J.R. Mathias, C.J. Hall, J.C. Emerson, P.S. Crosier, et al., *Pseudomonas aeruginosa* Type III secretion system interacts with phagocytes to modulate systemic infection of zebrafish embryos, Cell Microbiol. 11 (2009) 755–768, https://doi.org/10.1111/j.1462-5822.2009.01288.x.
[84] T.K. Prajsnar, R. Hamilton, J. Garcia-Lara, G. McVicker, A. Williams, M. Boots, et al., A privileged intraphagocyte niche is responsible for disseminated infection of *Staphylococcus aureus* in a zebrafish model, Cell Microbiol. 14 (2012) 1600–1619, https://doi.org/10.1111/j.1462-5822.2012.01826.x.
[85] T. Linnerz, C.J. Hall, The diverse roles of phagocytes during bacterial and fungal infections and sterile inflammation: lessons from zebrafish, Front. Immunol. 11 (2020), https://doi.org/10.3389/fimmu.2020.01094.
[86] S. Masud, V. Torraca, A.H. Meijer, Modeling infectious diseases in the context of a developing immune system, Curr. Top. Dev. Biol. 124 (2017) 277–329, https://doi.org/10.1016/bs.ctdb.2016.10.006.
[87] E. Colucci-Guyon, J.Y. Tinevez, S.A. Renshaw, P. Herbomel, Strategies of professional phagocytes in vivo: unlike macrophages, neutrophils engulf only surface-associated microbes, J. Cell Sci. 124 (Pt 18) (2011) 3053–3059, https://doi.org/10.1242/jcs.082792.
[88] T.K. Prajsnar, J.J. Serba, B.M. Dekker, J.F. Gibson, S. Masud, A. Fleming, et al., The autophagic response to *Staphylococcus aureus* provides an intracellular niche in neutrophils, Autophagy 17 (2021) 888–902, https://doi.org/10.1080/15548627.2020.1739443.
[89] V. Brinkmann, U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann, D.S. Weiss, et al., Neutrophil extracellular traps kill bacteria, Science 303 (2004) 1532–1535, https://doi.org/10.1126/science.1092385.
[90] H. Parker, A.M. Albrett, A.J. Kettle, C.C. Winterbourn, Myeloperoxidase associated with neutrophil extracellular traps is active and mediates bacterial killing in the presence of hydrogen peroxide, J. Leukoc. Biol. 91 (3) (2012) 369–376, https://doi.org/10.1189/jlb.0711387.
[91] D. Ermert, C.F. Urban, B. Laube, C. Goosmann, A. Zychlinsky, V. Brinkmann, Mouse neutrophil extracellular traps in microbial infections, J. Innate Immun. 1 (2009) 181–193, https://doi.org/10.1159/000205281.
[92] D.S.A. Abdallah, C. Lin, C.J. Ball, M.R. King, G.E. Duhamel, E.Y. Denkers, *Toxoplasma gondii* triggers release of human and mouse neutrophil extracellular traps, Infect. Immun. 80 (2012) 768–777, https://doi.org/10.1128/IAI.05730-11.

[93] R. Dowey, J. Cole, A.A.R. Thompson, R.C. Hull, C. Huang, J. Whatmore, et al., Enhanced neutrophil extracellular trap formation in COVID-19 is inhibited by the protein kinase C inhibitor ruboxistaurin, ERJ Open Res 8 (2022), https://doi.org/10.1183/23120541.00596-2021.

[94] D. Palić, J. Ostojić, C.B. Andreasen, J.A. Roth, Fish cast NETs: neutrophil extracellular traps are released from fish neutrophils, Dev. Comp. Immunol. 31 (2007) 805–816, https://doi.org/10.1016/j.dci.2006.11.010.

[95] D. Palić, C.B. Andreasen, J. Ostojić, R.M. Tell, J.A. Roth, Zebrafish (*Danio rerio*) whole kidney assays to measure neutrophil extracellular trap release and degranulation of primary granules, J. Immunol. Methods 319 (2007) 87–97, https://doi.org/10.1016/j.jim.2006.11.003.

[96] W. Chen, J. Zhao, D. Mu, Z. Wang, Q. Liu, Y. Zhang, et al., Pyroptosis mediates neutrophil extracellular trap formation during bacterial infection in zebrafish, J. Immunol. 206 (2021) 1913–1922, https://doi.org/10.4049/jimmunol.2001335.

[97] C.J. Johnson, J.M. Davis, A. Huttenlocher, J.F. Kernien, J.E. Nett, Emerging fungal pathogen *Candida auris* evades neutrophil attack, mBio 9 (2018), https://doi.org/10.1128/mBio.01403-18.

[98] B.G. Yipp, B. Petri, D. Salina, C.N. Jenne, B.N. Scott, L.D. Zbytnuik, et al., Infection-induced NETosis is a dynamic process involving neutrophil multitasking in vivo, Nat. Med. 18 (2012) 1386–1393, https://doi.org/10.1038/nm.2847.

[99] W. Zhao, D.K. Fogg, M.J. Kaplan, A novel image-based quantitative method for the characterization of NETosis, J. Immunol. Methods 423 (2015) 104–110, https://doi.org/10.1016/j.jim.2015.04.027.

[100] M. Faurschou, N. Borregaard, Neutrophil granules and secretory vesicles in inflammation, Microb. Infect. 5 (2003) 1317–1327, https://doi.org/10.1016/j.micinf.2003.09.008.

[101] E. Colucci-Guyon, A.S. Batista, S.D.S. Oliveira, M. Blaud, I.C. Bellettini, B. S. Marteyn, et al., Ultraspecific live imaging of the dynamics of zebrafish neutrophil granules by a histopermeable fluorogenic benzochalcone probe, Chem. Sci. 10 (2019) 3654, https://doi.org/10.1039/c8sc05593a.

[102] K.D. Buchan, T.K. Prajsnar, N.V. Ogryzko, N.W.M. de Jong, M. van Gent, J. Kolata, et al., A transgenic zebrafish line for in vivo visualisation of neutrophil myeloperoxidase, Epub 20190419, PLoS One 14 (4) (2019) e0215592, https://doi.org/10.1371/journal.pone.021559.

[103] M. Nguyen-Chi, Q.T. Phan, C. Gonzalez, J.F. Dubremetz, J.P. Levraud, G. Lutfalla, Transient infection of the zebrafish notochord with *E. coli* induces chronic inflammation, Dis Model Mech 7 (7) (2014) 871–882, https://doi.org/10.1242/dmm.014498.

[104] R. Medzhitov, Origin and physiological roles of inflammation, Nature 454 (7203) (2008) 428–435, https://doi.org/10.1038/nature07201.

[105] R. Medzhitov, Inflammation 2010: new adventures of an old flame, Cell 140 (6) (2010) 771–776, https://doi.org/10.1016/j.cell.2010.03.006.

[106] V.A. Fadok, D.L. Bratton, A. Konowal, P.W. Freed, J.Y. Westcott, P.M. Henson, Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGFbeta, PGE2, and PAF, J. Clin. Invest. 101 (4) (1998) 890–898, https://doi.org/10.1172/JCI1112.

[107] L.K. Cole, L.S. Ross, Apoptosis in the developing zebrafish embryo, Dev. Biol. 240 (1) (2001) 123–142, https://doi.org/10.1006/dbio.2001.0432.

[108] J.R. Mathias, B.J. Perrin, T.X. Liu, J. Kanki, A.T. Look, A. Huttenlocher, Resolution of inflammation by retrograde chemotaxis of neutrophils in transgenic zebrafish, Epub 20060908, J. Leukoc. Biol. 80 (6) (2006) 1281–1288, https://doi.org/10.1189/jlb.0506346.

[109] C.N. Serhan, J. Savill, Resolution of inflammation: the beginning programs the end, Nat. Immunol. 6 (12) (2005) 1191–1197, https://doi.org/10.1038/ni1276.

[110] C.D. Buckley, D.W. Gilroy, C.N. Serhan, Proresolving lipid mediators and mechanisms in the resolution of acute inflammation, Immunity 40 (3) (2014) 315–327, https://doi.org/10.1016/j.immuni.2014.02.009.

[111] D.L. Bratton, P.M. Henson, Neutrophil clearance: when the party is over, cleanup begins, Epub 20110723, Trends Immunol. 32 (8) (2011) 350–357, https://doi.org/10.1016/j.it.2011.04.009.

[112] C.A. Loynes, J.S. Martin, A. Robertson, D.M.I. Trushell, P.W. Ingham, M.K. B. Whyte, et al., Pivotal Advance: pharmacological manipulation of inflammation resolution during spontaneously resolving tissue neutrophilia in the zebrafish, J. Leukoc. Biol. 87 (2) (2010) 203–212, https://doi.org/10.1189/jlb.0409255.

[113] A. Kaveh, F.A. Bruton, C. Buckley, M.E.M. Oremek, C.S. Tucker, J.J. Mullins, et al., Live imaging of heart injury in larval zebrafish reveals a multi-stage model of neutrophil and macrophage migration, Epub 20201019, Front. Cell Dev. Biol. 8 (2020) 579943, https://doi.org/10.3389/fcell.2020.579943.

[114] C.D. Lucas, K.C. Allen, D.A. Dorward, L.J. Hoodless, L.A. Melrose, J.A. Marwick, et al., Flavones induce neutrophil apoptosis by down-regulation of Mcl-1 via a proteasomal-dependent pathway, Epub 20121129, Faseb. J. 27 (3) (2013) 1084–1094, https://doi.org/10.1096/fj.12-218990.

[115] N.V. Ogryzko, A. Lewis, H.L. Wilson, A.H. Meijer, S.A. Renshaw, P.M. Elks, Hif-1α-Induced expression of Il-1β protects against mycobacterial infection in zebrafish, Epub 20181214, J. Immunol. 202 (2) (2019) 494–502, https://doi.org/10.4049/jimmunol.1801139.

[116] A.A.R. Thompson, P.M. Elks, H.M. Marriott, S. Eamsamarng, K.R. Higgins, A. Lewis, et al., Hypoxia-inducible factor 2α regulates key neutrophil functions in humans, mice, and zebrafish, Epub 20131106, Blood 123 (3) (2014) 366–376, https://doi.org/10.1182/blood-2013-05-500207.

[117] Y. Schild, A. Mohamed, E.J. Wootton, A. Lewis, P.M. Elks, Hif-1alpha stabilisation is protective against infection in zebrafish comorbid models, Epub 20200618, FEBS J. 287 (18) (2020) 3925–3943, https://doi.org/10.1111/febs.15433.

[118] A.L. Robertson, N.V. Ogryzko, K.M. Henry, C.A. Loynes, M.J. Foulkes, M. M. Meloni, et al., Identification of benzopyrone as a common structural feature in compounds with anti-inflammatory activity in a zebrafish phenotypic screen, Epub 20160414, Dis Model Mech 9 (6) (2016) 621–632, https://doi.org/10.1242/dmm.024935.

[119] N. Andrews, M.C. Ramel, S. Kumar, Y. Alexandrov, D.J. Kelly, S.C. Warren, et al., Visualising apoptosis in live zebrafish using fluorescence lifetime imaging withoptical projection tomography to map FRET biosensor activity in space and time, Epub 20160111, J. Biophot. 9 (4) (2016) 414–424, https://doi.org/10.1002/jbio.201500258.

[120] A.L. Robertson, G.R. Holmes, A.N. Bojarczuk, J. Burgon, C.A. Loynes, M. Chimen, et al., A zebrafish compound screen reveals modulation of neutrophil reverse migration as an anti-inflammatory mechanism, Sci. Transl. Med. 6 (225) (2014) 225ra29, https://doi.org/10.1126/scitranslmed.3007672.

[121] P.M. Elks, F.J. van Eeden, G. Dixon, X. Wang, C.C. Reyes-Aldasoro, P.W. Ingham, et al., Activation of hypoxia-inducible factor-1α (Hif-1α) delays inflammation resolution by reducing neutrophil apoptosis and reverse migration in a zebrafish inflammation model, Epub 20110509, Blood 118 (3) (2011) 712–722, https://doi.org/10.1182/blood-2010-12-324186.

[122] S. Nourshargh, S.A. Renshaw, B.A. Imhof, Reverse migration of neutrophils: where, when, how, and why?, Epub 20160404, Trends Immunol. 37 (5) (2016) 273–286, https://doi.org/10.1016/j.it.2016.03.006.

[123] A. Woodfin, M.B. Voisin, M. Beyrau, B. Colom, D. Caille, F.M. Diapouli, et al., The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo, Epub 20110626, Nat. Immunol. 12 (8) (2011) 761–769, https://doi.org/10.1038/ni.2062.

[124] B. Colom, J.V. Bodkin, M. Beyrau, A. Woodfin, C. Ody, C. Rourke, et al., Leukotriene B4-neutrophil elastase axis drives neutrophil reverse transendothelial cell migration in vivo, Epub 20150602, Immunity 42 (6) (2015) 1075–1086, https://doi.org/10.1016/j.immuni.2015.05.010.

[125] C.D. Buckley, E.A. Ross, H.M. McGettrick, C.E. Osborne, O. Haworth, C. Schmutz, et al., Identification of a phenotypically and functionally distinct population of long-lived neutrophils in a model of reverse endothelial migration, Epub 20051205, J. Leukoc. Biol. 79 (2) (2006) 303–311, https://doi.org/10.1189/jlb.0905496.

[126] D. Irimia, F. Ellett, Big insights from small volumes: deciphering complex leukocyte behaviors using microfluidics, Epub 20160518, J. Leukoc. Biol. 100 (2) (2016) 291–304, https://doi.org/10.1189/jlb.5RU0216-056R.

[127] G.R. Holmes, S.R. Anderson, G. Dixon, A.L. Robertson, C.C. Reyes-Aldasoro, S. A. Billings, et al., Repelled from the wound, or randomly dispersed? Reverse migration behaviour of neutrophils characterized by dynamic modelling, Epub 20120905, J. R. Soc., Interface 9 (77) (2012) 3229–3239, https://doi.org/10.1098/rsif.2012.0542.

[128] F. Ellett, P.M. Elks, A.L. Robertson, N.V. Ogryzko, S.A. Renshaw, Defining the phenotype of neutrophils following reverse migration in zebrafish, Epub 20150612, J. Leukoc. Biol. 98 (6) (2015) 975–981, https://doi.org/10.1189/jlb.3MA0315-105R.

[129] S.K. Yoo, A. Huttenlocher, Spatiotemporal photolabeling of neutrophil trafficking during inflammation in live zebrafish, Epub 20110119, J. Leukoc. Biol. 89 (5) (2011) 661–667, https://doi.org/10.1189/jlb.1010567.

[130] S. Tauzin, T.W. Starnes, F.B. Becker, P.Y. Lam, A. Huttenlocher, Redox and Src family kinase signaling control leukocyte wound attraction and neutrophil reverse migration, J. Cell Biol. 207 (5) (2014) 589–598, https://doi.org/10.1083/jcb.201408090.

[131] C.A. Loynes, J.A. Lee, A.L. Robertson, M.J. Steel, F. Ellett, Y. Feng, et al., PGE2 production at sites of tissue injury promotes an anti-inflammatory neutrophil phenotype and determines the outcome of inflammation resolution in vivo, Epub 20180905, Sci. Adv. 4 (9) (2018), https://doi.org/10.1126/sciadv.aar8320.

[132] H.M. Isles, K.D. Herman, A.L. Robertson, C.A. Loynes, L.R. Prince, P.M. Elks, et al., The CXCL12/CXCR4 signaling Axis retains neutrophils at inflammatory sites in zebrafish, Epub 20190731, Front. Immunol. 10 (2019) 1784, https://doi.org/10.3389/fimmu.2019.01784.

[133] S. de Oliveira, C.C. Reyes-Aldasoro, S. Candel, S.A. Renshaw, V. Mulero, A. Calado, Cxcl8 (IL-8) mediates neutrophil recruitment and behavior in the zebrafish inflammatory response, Epub 20130318, J. Immunol. 190 (8) (2013) 4349–4359, https://doi.org/10.4049/jimmunol.1203266.

[134] S. Xu, F. Xie, L. Tian, S.H. Manno, F.A.M. Manno, S.H. Cheng, Prolonged neutrophil retention in the wound impairs zebrafish heart regeneration after cryoinjury, Epub 20190914, Fish Shellfish Immunol. 94 (2019) 447–454, https://doi.org/10.1016/j.fsi.2019.09.030.

[135] D. Wu, Y. Zeng, Y. Fan, J. Wu, T. Mulatibieke, J. Ni, et al., Reverse-migrated neutrophils regulated by JAM-C are involved in acute pancreatitis-associated lung injury, Epub 20160204, Sci. Rep. 6 (2016) 20545, https://doi.org/10.1038/srep20545.

[136] L.v.G. Jørgensen, The fish parasite *Ichthyophthirius multifiliis* - host immunology, vaccines and novel treatments, Epub 2017/06/17, Fish Shellfish Immunol. 67 (2017) 586–595, https://doi.org/10.1016/j.fsi.2017.06.044.

[137] H. Mathiessen, S. Kjeldgaard-Nintemann, C.M.F. Gonzalez, C. Henard, J. A. Reimer, S.V. Gelskov, et al., Acute immune responses in zebrafish and evasive behavior of a parasite - who is winning? Front. Cell. Infect. Microbiol. 13 (2023) https://doi.org/10.3389/fcimb.2023.1190931.

[138] R.A. Matthews, *Ichthyophthirius multifiliis* fouquet and ichthyophthiriosis in freshwater teleosts, Adv. Parasitol. 59 (2005) 159–241, https://doi.org/10.1016/S0065-308X(05)59003-1.

[139] H. Dickerson, T. Clark, *Ichthyophthirius multifiliis*: a model of cutaneous infection and immunity in fishes, Immunol. Rev. 166 (1998) 377–384, https://doi.org/10.1111/j.1600-065x.1998.tb01277.x. PubMed PMID: 9914927.

[140] S.F. Gonzalez, K. Buchmann, M.E. Nielsen, Complement expression in common carp (*Cyprinus carpio* L.) during infection with *Ichthyophthirius multifiliis*, Dev. Comp. Immunol. 31 (6) (2007) 576–586, https://doi.org/10.1016/j.dci.2006.08.010.

[141] S.F. Gonzalez, K. Buchmann, M.E. Nielsen, *Ichthyophthirius multifiliis* infection induces massive up-regulation of serum amyloid A in carp (*Cyprinus carpio*), Vet. Immunol. Immunopathol. 115 (1–2) (2007) 172–178, https://doi.org/10.1016/j.vetimm.2006.09.007.

[142] S.F. Gonzalez, K. Buchmann, M.E. Nielsen, Real-time gene expression analysis in carp (*Cyprinus carpio* L.) skin: inflammatory responses caused by the ectoparasite *Ichthyophthirius multifiliis*, Fish Shellfish Immunol. 22 (6) (2007) 641–650, https://doi.org/10.1016/j.fsi.2006.08.011.

[143] M.A. Burkart, T.G. Clark, H.W. Dickerson, Immunisation of channel catfish, *Ictalurus-punctatus* Rafinesque, againt *Ichthyophthirius multifiliis* (Fouquet) - killed versus live vaccine, J. Fish. Dis. 13 (5) (1990) 401–410, https://doi.org/10.1111/j.1365-2761.1990.tb00799.x.

[144] T.G. Clark, H.W. Dickerson, J.B. Gratzek, R.C. Findly, *In vitro* response of *Ichthyophthirius multifiliis* to sera from immune channel catfish, J. Fish. Biol. 31 (1987) 203–208, https://doi.org/10.1111/j.1095-8649.1987.tb05314.x. PubMed PMID: WOS:A1987L621200031.

[145] T.G. Clark, H.W. Dickerson, R.C. Findly, Immune response of channel catfish to ciliary antigens of *Ichthyophthirius multifiliis*, Dev. Comp. Immunol. 12 (3) (1988) 581–594, https://doi.org/10.1016/0145-305X(88)90074-2.

[146] T.G. Clark, T.L. Lin, H.W. Dickerson, Surface antigen cross-linking triggers forced exit of a protozoan parasite from its host, Proc. Natl. Acad. Sci. U.S.A. 93 (13) (1996) 6825–6829, https://doi.org/10.1073/pnas.93.13.6825.

[147] L.v.G. Jørgensen, J. Sigh, P.W. Kania, L. Holten-Andersen, K. Buchmann, T. Clark, et al., Approaches towards DNA vaccination against a skin ciliate parasite in fish, PLoS One 7 (11) (2012), https://doi.org/10.1371/journal.pone.0048129.

[148] L.v.G. Jørgensen, R. Korbut, S. Jeberg, P.W. Kania, K. Buchmann, Association between adaptive immunity and neutrophil dynamics in zebrafish (*Danio rerio*) infected by a parasitic ciliate, PLoS One (2018), https://doi.org/10.1371/journal.pone.0203297.

[149] D. Gomez, Z. Xu, L.v.G. Jørgensen, K. Buchmann, J.O. Sunyer, Orchestrated interaction between IgT and complement C3 to control a skin parasite of rainbow trout, Fish Shellfish Immunol. 34 (6) (2013) 1708.

[150] L.v.G. Jørgensen, E. Nemli, R.D. Heinecke, M.K. Raida, K. Buchmann, Immunerelevant genes expressed in rainbow trout following immunisation with a live vaccine against Ichthyophthirius multifiliis, Dis. Aquat. Org. 80 (3) (2008) 189–197, https://doi.org/10.3354/dao01935.

[151] L.v.G. Jørgensen, R.D. Heinecke, K. Skjødt, K.J. Rasmussen, K. Buchmann, Experimental evidence for direct *in situ* binding of IgM and IgT to early trophonts of *Ichthyophthirius multifiliis* (Fouquet) in the gills of rainbow trout, *Oncorhynchus mykiss* (Walbaum), J. Fish. Dis. 34 (10) (2011) 749–755, https://doi.org/10.1111/j.1365-2761.2011.01291.x.

[152] L.v.G. Jørgensen, The dynamics of neutrophils in zebrafish (*Danio rerio*) during infection with the parasite *Ichthyophthirius multifiliis*, Fish Shellfish Immunol. 55 (2016) 159–164, https://doi.org/10.1016/j.fsi.2016.05.026.

[153] L.v.G. Jørgensen, P.W. Kania, K.J. Rasmussen, A.H. Mattsson, J. Schmidt, A. Al-Jubury, et al., Rainbow trout (*Oncorhynchus mykiss*) immune response towards a recombinant vaccine targeting the parasitic ciliate *Ichthyophthirius multifiliis*, J. Fish. Dis. 40 (2017) 1815–1821, https://doi.org/10.1111/jfd.12653.

[154] Z. Xu, D. Parra, D. Gomez, I. Salinas, Y.A. Zhang, L.v.G. Jørgensen, et al., Teleost skin, an ancient mucosal surface that elicits gut-like immune responses, Proc. Natl. Acad. Sci. U.S.A. 110 (32) (2013) 13097–13102, https://doi.org/10.1073/pnas.1304319110.

[155] M.L. Cross, R.A. Matthews, Ichthyophthiriasis in carp, *Cyprinus carpio* L.: fate of parasites in immunized fish, J. Fish. Dis. 15 (6) (1992) 497–505, https://doi.org/10.1111/j.1365-2761.1992.tb00681.x.

[156] M.L. Cross, R.A. Matthews, Localized leukocyte response to *Ichthyophthirius multifiliis* establishment in immune carp *Cyprinus carpio* L, Vet. Immunol. Immunopathol. 38 (3–4) (1993) 341–358, https://doi.org/10.1016/0165-2427(93)90092-i.

[157] D.H. Xu, P.H. Klesius, C.A. Shoemaker, Protective immunity of Nile tilapia against *Ichthyophthirius multifiliis* post-immunization with live theronts and sonicated trophonts, Fish Shellfish Immunol. 25 (1–2) (2008) 124–127, https://doi.org/10.1016/j.fsi.2008.03.012.

[158] D.H. Xu, G.S.A. Moreira, C.A. Shoemaker, D. Zhang, B.H. Beck, Expression of immune genes in systemic and mucosal immune tissues of channel catfish vaccinated with live theronts of *Ichthyophthirius multifiliis*, Fish Shellfish Immunol. 66 (2017) 540–547, https://doi.org/10.1016/j.fsi.2017.05.051.

[159] X. Wang, T.G. Clark, J. Noe, H.W. Dickerson, Immunisation of channel catfish, *Ictalurus punctatus*, with *Ichthyophthirius multifiliis* immobilisation antigens elicits serotype-specific protection, Fish Shellfish Immunol. 13 (5) (2002) 337–350, https://doi.org/10.1006/fsim.2001.0410.

[160] D.H. Xu, P.H. Klesius, C.A. Shoemaker, Effect of immunization of channel catfish with inactivated trophonts on serum and cutaneous antibody titers and survival against *Ichthyophthirius multifiliis*, Fish Shellfish Immunol. 26 (4) (2009) 614–618, https://doi.org/10.1016/j.fsi.2008.09.015.

[161] D.H. Xu, Q.Z. Zhang, C.A. Shoemaker, D.H. Zhang, G.S.A. Moreira, Molecular immune response of channel catfish immunized with live theronts of *Ichthyophthirius multifiliis*, Fish Shellfish Immunol. 54 (2016) 86–92, https://doi.org/10.1016/j.fsi.2016.03.166.

[162] M.L. Cross, Localized cellular responses to *Ichthyophthirius multifiliis*: protection or pathogenesis? Parasitol. Today 10 (9) (1994) 364–368, https://doi.org/10.1016/0169-4758(94)90253-4.

[163] M.M. Olsen, P.W. Kania, R.D. Heinecke, K. Skjoedt, K.J. Rasmussen, K. Buchmann, Cellular and humoral factors involved in the response of rainbow trout gills to *Ichthyophthirius multifiliis* infections: molecular and immunohistochemical studies, Fish Shellfish Immunol. 30 (3) (2011) 859–869, https://doi.org/10.1016/j.fsi.2011.01.010.

[164] K. Syahputra, P.W. Kania, A. Al-Jubury, H. Marnis, A.C. Setyawan, K. Buchmann, Differential immune gene response in gills, skin, and spleen of rainbow trout *Oncorhynchus mykiss* infected by *Ichthyophthirius multifiliis*, Epub 2019/06/20, PLoS One 14 (6) (2019) e0218630, https://doi.org/10.1371/journal.pone.0218630.

[165] R.S. Hines, D.T. Spira, Ichthyophthiriasis in mirror carp *Cyprinus carpio* (L). III. Pathology &amp, J. Fish. Biol. 6 (2) (1974) 189, https://doi.org/10.1111/j.1095-8649.1974.tb04536.x. PubMed PMID: WOS: A1974S413600008.

[166] M.T. Ventura, I. Paperna, Histopathology of *Ichthyophthirius multifiliis* infections in fishes, J. Fish. Biol. 27 (2) (1985) 185–203, https://doi.org/10.1111/j.1095-8649.1985.tb04020.x.

[167] G.S. Moreira, C.A. Shoemaker, D. Zhang, D.H. Xu, Expression of immune genes in skin of channel catfish immunized with live theronts of Ichthyophthirius multifiliis, Epub 2016/11/02, Parasite Immunol. 39 (1) (2017), https://doi.org/10.1111/pim.12397.

[168] G.H. Sack Jr., Serum amyloid A - a review, Epub 20180830, Mol. Med. 24 (1) (2018) 46, https://doi.org/10.1186/s10020-018-0047-0.

[169] F. Tian, C. Tong, C. Feng, K. Wanghe, K. Zhao, Transcriptomic profiling of Tibetan highland fish (*Gymnocypris przewalskii*) in response to the infection of parasite ciliate *Ichthyophthirius multifiliis*, Epub 2017/09/09, Fish Shellfish Immunol. 70 (2017) 524–535, https://doi.org/10.1016/j.fsi.2017.09.003.

[170] R. Jaafar, J. Ødegard, H. Mathiessen, A.M. Karami, M.H. Marana, L.v. G. Jørgensen, et al., Quantitative trait loci (QTL) associated with resistance of rainbow trout *Oncorhynchus mykiss* against the parasitic ciliate *Ichthyophthirius multifiliis*, Epub 2020/09/19, J. Fish. Dis. 43 (12) (2020) 1591–1602, https://doi.org/10.1111/jfd.13264.

[171] S. Hong, J. Zou, M. Crampe, S. Peddie, G. Scapigliati, N. Bols, et al., The production and bioactivity of rainbow trout (*Oncorhynchus mykiss*) recombinant IL-1β, Vet. Immunol. Immunopathol. 81 (1–2) (2001) 14, https://doi.org/10.1016/S0165-2427(01)00328-2.

[172] T. Wang, K. Mai, Q. Ai, A review of cyclooxygenase-2 role in fish, Austin Journal of Nutrition and Metabolism 3 (1) (2016) 6.

[173] J. Wu, Y.H. Shi, X.H. Zhang, G.H. Li, M. Li, J. Chen, Molecular characterization of an IL-1β gene from the large yellow croaker (*Larimichthys crocea*) and its effect on fish defense against *Vibrio alginolyticus* infection, Zool. Res. 36 (2015) 8.

[174] R.D. Heinecke, K. Buchmann, Inflammatory response of rainbow trout Oncorhynchus mykiss (Walbaum, 1792) larvae against Ichthyophthirius multifiliis, Epub 2012/12/25, Fish Shellfish Immunol. 34 (2) (2013) 521–528, https://doi.org/10.1016/j.fsi.2012.11.036.

[175] K. Syahputra, P.W. Kania, A. Al-Jubury, R.M. Jafaar, R.P. Dirks, K. Buchmann, Transcriptomic analysis of immunity in rainbow trout (*Oncorhynchus mykiss*) gills infected by *Ichthyophthirius multifiliis*, Epub 2018/12/05, Fish Shellfish Immunol. 86 (2019) 486–496, https://doi.org/10.1016/j.fsi.2018.11.075.

[176] J. Feng, T. Han, Y. Zhang, B. Zhang, D. Huang, T. Wang, et al., Molecular characterization and biological function of CXCR1 in Nocardia seriolae-infected largemouth bass (*Micropterus salmoides*), Epub 20210421, Tissue Cell 72 (2021) 101551, https://doi.org/10.1016/j.tice.2021.101551.

[177] F. Zhao, Y.W. Li, H.J. Pan, C.B. Shi, X.C. Luo, A.X. Li, et al., Expression profiles of toll-like receptors in channel catfish (*Ictalurus punctatus*) after infection with *Ichthyophthirius multifiliis*, Epub 2013/06/08, Fish Shellfish Immunol. 35 (3) (2013) 993–997, https://doi.org/10.1016/j.fsi.2013.05.023.

[178] T. Kawai, S. Akira, The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors, Epub 20100420, Nat. Immunol. 11 (5) (2010) 373–384, https://doi.org/10.1038/ni.1863.

[179] M. Landstrom, The TAK1-TRAF6 signalling pathway, Epub 20100112, Int. J. Biochem. Cell Biol. 42 (5) (2010) 585–589, https://doi.org/10.1016/j.biocel.2009.12.023.

[180] J. Gohda, T. Matsumura, J. Inoue, Cutting edge: TNFR-associated factor (TRAF) 6 is essential for MyD88-dependent pathway but not toll/IL-1 receptor domaincontaining adaptor-inducing IFN-beta (TRIF)-dependent pathway in TLR signaling, J. Immunol. 173 (5) (2004) 2913–2917, https://doi.org/10.4049/jimmunol.173.5.2913.

[181] M. Shen, Z. Jiang, K. Zhang, C. Li, F. Liu, Y. Hu, et al., Transcriptome analysis of grass carp (*Ctenopharyngodon idella*) and Holland's spinibarbel (*Spinibarbus hollandi*) infected with *Ichthyophthirius multifiliis*, Fish Shellfish Immunol. 121 (2022) 305–315, https://doi.org/10.1016/j.fsi.2022.01.008.

[182] A. Kawakami, T. Fukazawa, H. Takeda, Early fin primordia of zebrafish larvae regenerate by a similar growth control mechanism with adult regeneration, Dev. Dynam. 231 (4) (2004) 693–699, https://doi.org/10.1002/dvdy.20181.

[183] L. Cavone, T. McCann, L.K. Drake, E.A. Aguzzi, A.M. Oprişoreanu, E. Pedersen, et al., A unique macrophage subpopulation signals directly to progenitor cells to promote regenerative neurogenesis in the zebrafish spinal cord, e6. Epub 20210524, Dev. Cell 56 (11) (2021) 1617–1630, https://doi.org/10.1016/j.devcel.2021.04.031.

[184] M. Nguyen-Chi, P. Luz-Crawford, L. Balas, T. Sipka, R. Contreras-López, A. Barthelaix, et al., Pro-resolving mediator protectin D1 promotes epimorphic regeneration by controlling immune cell function in vertebrates, Epub 20200706,

Br. J. Pharmacol. 177 (17) (2020) 4055–4073, https://doi.org/10.1111/bph.15156.
[185] J. Shao, X. Qian, C. Zhang, Z. Xu, Fin regeneration from tail segment with musculature, endoskeleton, and scales, J. Exp. Zool. B Mol. Dev. Evol. 312 (7) (2009) 762–769, https://doi.org/10.1002/jez.b.21295.
[186] L. Li, B. Yan, Y.Q. Shi, W.Q. Zhang, Z.L. Wen, Live imaging reveals differing roles of macrophages and neutrophils during zebrafish tail fin regeneration, Epub 20120509, J. Biol. Chem. 287 (30) (2012) 25353–25360, https://doi.org/10.1074/jbc.M112.349126.
[187] J. Shao, D. Chen, Q. Ye, J. Cui, Y. Li, L. Li, Tissue regeneration after injury in adult zebrafish: the regenerative potential of the caudal fin, Dev. Dynam. 240 (5) (2011) 1271–1277, https://doi.org/10.1002/dvdy.2260.
[188] M.C. Keightley, C.H. Wang, V. Pazhakh, G.J. Lieschke, Delineating the roles of neutrophils and macrophages in zebrafish regeneration models, Epub 20140721, Int. J. Biochem. Cell Biol. 56 (2014) 92–106, https://doi.org/10.1016/j.biocel.2014.07.010.
[189] T.A. Fuchs, U. Abed, C. Goosmann, R. Hurwitz, I. Schulze, V. Wahn, et al., Novel cell death program leads to neutrophil extracellular traps, Epub 20070108, J. Cell Biol. 176 (2) (2007) 231–241, https://doi.org/10.1083/jcb.200606027.
[190] K.L. Rock, E. Latz, F. Ontiveros, H. Kono, The sterile inflammatory response, Annu. Rev. Immunol. 28 (2010) 321–342, https://doi.org/10.1146/annurevimmunol-030409-101311.
[191] M. Nguyen-Chi, B. Laplace-Builhé, J. Travnickova, P. Luz-Crawford, G. Tejedor, G. Lutfalla, et al., TNF signaling and macrophages govern fin regeneration in zebrafish larvae, Epub 20170810, Cell Death Dis. 8 (8) (2017) e2979, https://doi.org/10.1038/cddis.2017.374.
[192] P. Herbomel, B. Thisse, C. Thisse, Ontogeny and behaviour of early macrophages in the zebrafish embryo, Development 126 (17) (1999) 3735–3745, https://doi.org/10.1242/dev.126.17.3735.
[193] E.S. Trombetta, I. Mellman, Cell biology of antigen processing *in vitro* and *in vivo*, Annu. Rev. Immunol. 23 (2005) 975–1028, https://doi.org/10.1146/annurev.immunol.22.012703.104538.
[194] G. Lugo-Villarino, K.M. Balla, D.L. Stachura, K. Bañuelos, M.B.F. Werneck, D. Traver, Identification of dendritic antigen-presenting cells in the zebrafish, Epub 20100823, Proc. Natl. Acad. Sci. U.S.A. 107 (36) (2010) 15850–15855, https://doi.org/10.1073/pnas.1000494107.
[195] F.M. Keij, B.E.V. Koch, F.L. Vigario, S.H.P. Simons, J.G.C. van Hasselt, H.R. Taal, et al., Zebrafish larvae as experimental model to expedite the search for new biomarkers and treatments for neonatal sepsis, Epub 20210618, J Clin Transl Sci 5 (1) (2021) e140.
[196] Q. Zhou, C. Zhao, Z. Yang, R. Qu, Y. Li, Y. Fan, et al., Cross-organ single-cell transcriptome profiling reveals macrophage and dendritic cell heterogeneity in zebrafish, Epub 20230714, Cell Rep. 42 (7) (2023) 112793, https://doi.org/10.1016/j.celrep.2023.112793.
[197] K.Z. Miao, G.Y. Kim, G.K. Meara, X. Qin, H. Feng, Tipping the scales with zebrafish to understand adaptive tumor immunity, Front. Cell Dev. Biol. 9 (2021), https://doi.org/10.3389/fcell.2021.660969.
[198] A. Khan, V.K. Singh, R.L. Hunter, C. Jagannath, Macrophage heterogeneity and plasticity in tuberculosis, J. Leukoc. Biol. 106 (2) (2019) 275–282, https://doi.org/10.1002/JLB.MR0318-095RR.
[199] W. Noël, G. Raes, G.H. Ghassabeh, P. De Baetselier, A. Beschin, Alternatively activated macrophages during parasite infections, Trends Parasitol. 20 (3) (2004) 126–133, https://doi.org/10.1016/j.pt.2004.01.004.
[200] C.D. Mills, K. Ley, M1 and M2 macrophages: the chicken and the egg of immunity, J. Innate Immun. 6 (6) (2014) 716–726, https://doi.org/10.1159/000364945.
[201] A. Bojarczuk, K.A. Miller, R. Hotham, A. Lewis, N.V. Ogryzko, A.A. Kamuyango, et al., *Cryptococcus neoformans* intracellular proliferation and capsule size determines early macrophage control of infection, Epub 20160218, Sci. Rep. 6 (2016) 21489, https://doi.org/10.1038/srep21489.
[202] E.M. Walton, M.R. Cronan, R.W. Beerman, D.M. Tobin, The macrophagespecific promoter mfap4 allows live, long-term analysis of macrophage behavior during mycobacterial infection in zebrafish, PLoS One 10 (10) (2015) e0138949, https://doi.org/10.1371/journal.pone.0138949.
[203] M. Nguyen-Chi, B. Laplace-Builhe, J. Travnickova, P. Luz-Crawford, G. Tejedor, Q.T. Phan, et al., Identification of polarized macrophage subsets in zebrafish, Elife 4 (2015) e07288, https://doi.org/10.7554/eLife.07288.
[204] J. Rougeot, V. Torraca, A. Zakrzewska, Z. Kanwal, H.J. Jansen, F. Sommer, et al., RNAseq profiling of leukocyte populations in zebrafish larvae reveals a cxcl11 chemokine gene as a marker of macrophage polarization during mycobacterial infection, Front. Immunol. 10 (2019), https://doi.org/10.3389/fimmu.2019.00832.
[205] M. Guilliams, J. Bonnardel, B. Haest, B. Vanderborght, C. Wagner, A. Remmerie, et al., Spatial proteogenomics reveals distinct and evolutionarily conserved hepatic macrophage niches, e38. Epub 20220111, Cell 185 (2) (2022) 379–396, https://doi.org/10.1016/j.cell.2021.12.018.
[206] J. Mazzolini, S. Le Clerc, G. Morisse, C. Coulonges, L.E. Kuil, T.J. van Ham, et al., Gene expression profiling reveals a conserved microglia signature in larval zebrafish, Glia 68 (2) (2020) 298–315, https://doi.org/10.1002/glia.23717.
[207] S. Wu, L.T.M. Nguyen, H. Pan, S. Hassan, Y. Dai, J. Xu, et al., Two phenotypically and functionally distinct microglial populations in adult zebrafish, Sci. Adv. 6 (47) (2020) eabd1160, https://doi.org/10.1126/sciadv.abd1160.
[208] C. Bohaud, M.D. Johansen, C. Jorgensen, N. Ipseiz, L. Kremer, F. Djouad, The role of macrophages during zebrafish injury and tissue regeneration under infectious and non-infectious conditions, Epub 20210721, Front. Immunol. 12 (2021) 707824, https://doi.org/10.3389/fimmu.2021.707824.
[209] N.J. Silva, L.C. Dorman, I.D. Vainchtein, N.C. Horneck, A.V. Molofsky, In situ and transcriptomic identification of microglia in synapse-rich regions of the developing zebrafish brain, Nat. Commun. 12 (1) (2021) 5916, https://doi.org/10.1038/s41467-021-26206-x.
[210] B.R. Moyse, R.J. Richardson, A population of injury-responsive lymphoid cells expresses mpeg1.1 in the adult zebrafish heart, ImmunoHorizons 4 (8) (2020) 464–474, https://doi.org/10.4049/immunohorizons.2000063.
[211] C.J. Cambier, K.K. Takaki, R.P. Larson, R.E. Hernandez, D.M. Tobin, K.B. Urdahl, et al., Mycobacteria manipulate macrophage recruitment through coordinated use of membrane lipids, Epub 20131215, Nature 505 (7482) (2014) 218–222, https://doi.org/10.1038/nature12799.
[212] C.J. Cambier, S.M. O'Leary, M.P. O'Sullivan, J. Keane, L. Ramakrishnan, Phenolic glycolipid facilitates mycobacterial escape from microbicidal tissueresident macrophages, e4. Epub 20170824, Immunity 47 (3) (2017) 552–565, https://doi.org/10.1016/j.immuni.2017.08.003.
[213] F. Sommer, N.V. Ortiz Zacarías, L.H. Heitman, A.H. Meijer, Inhibition of macrophage migration in zebrafish larvae demonstrates in vivo efficacy of human CCR2 inhibitors, Dev. Comp. Immunol. 116 (2021) 103932, https://doi.org/10.1016/j.dci.2020.103932.
[214] R.A. Morales, M.L. Allende, Peripheral macrophages promote tissue regeneration in zebrafish by fine-tuning the inflammatory response, Epub 20190215, Front. Immunol. 10 (2019) 253, https://doi.org/10.3389/fimmu.2019.00253.
[215] V. Torraca, S. Masud, H.P. Spaink, A.H. Meijer, Macrophage-pathogen interactions in infectious diseases: new therapeutic insights from the zebrafish host model, Dis Model Mech 7 (7) (2014) 785–797, https://doi.org/10.1242/dmm.015594.
[216] P. Herbomel, B. Thisse, C. Thisse, Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a MCSF receptor-dependent invasive process, Dev. Biol. 238 (2) (2001) 274–288, https://doi.org/10.1006/dbio.2001.0393.
[217] E. Murayama, K. Kissa, A. Zapata, E. Mordelet, V. Briolat, H.F. Lin, et al., Tracing hematopoietic precursor migration to successive hematopoietic organs during zebrafish development, Immunity 25 (6) (2006) 963–975, https://doi.org/10.1016/j.immuni.2006.10.015.
[218] C.E. Shiau, Z. Kaufman, A.M. Meireles, W.S. Talbot, Differential requirement for irf8 in formation of embryonic and adult macrophages in zebrafish, PLoS One 10 (1) (2015) e0117513, https://doi.org/10.1371/journal.pone.0117513.
[219] D.L. Stachura, D. Traver, Cellular dissection of zebrafish hematopoiesis, Methods Cell Biol. 101 (2011) 75–110, https://doi.org/10.1016/b978-0-12-387036-0.00004-9.
[220] G. Ferrero, C.B. Mahony, E. Dupuis, L. Yvernogeau, E. Di Ruggiero, M. Miserocchi, et al., Embryonic microglia derive from primitive macrophages and are replaced by cmyb-dependent definitive microglia in zebrafish, Cell Rep. 24 (1) (2018) 130–141, https://doi.org/10.1016/j.celrep.2018.05.066.
[221] N. Ranawat, I. Masai, Mechanisms underlying microglial colonization of developing neural retina in zebrafish, Elife 10 (2021) e70550, https://doi.org/10.7554/eLife.70550.
[222] F. Peri, C. Nüsslein-Volhard, Live imaging of neuronal degradation by microglia reveals a role for v0-ATPase a1 in phagosomal fusion *in vivo*, Cell 133 (5) (2008) 916–927, https://doi.org/10.1016/j.cell.2008.04.037.
[223] H.A. Rutherford, A. Clarke, E.V. Chambers, J.J. Petts, E.G. Carson, H.M. Isles, et al., A zebrafish reporter line reveals immune and neuronal expression of endogenous retrovirus, Epub 20220413, Dis Model Mech 15 (4) (2022), https://doi.org/10.1242/dmm.048921.
[224] M. Olah, V. Menon, N. Habib, M.F. Taga, Y. Ma, C.J. Yung, et al., Single cell RNA sequencing of human microglia uncovers a subset associated with Alzheimer' s disease, Nat. Commun. 11 (1) (2020) 6129, https://doi.org/10.1038/s41467-020-19737-2.
[225] G. Ferrero, E. Gomez, S. Lyer, M. Rovira, M. Miserocchi, D.M. Langenau, et al., The macrophage-expressed gene (mpeg) 1 identifies a subpopulation of B cells in the adult zebrafish, Epub 20200107, J. Leukoc. Biol. 107 (3) (2020) 431–443, https://doi.org/10.1002/jlb.1a1119-223r.
[226] X. Lin, Q. Zhou, G. Lin, C. Zhao, Z. Wen, Endoderm-derived myeloid-like metaphocytes in zebrafish gill mediate soluble antigen-induced immunity, Cell Rep. 33 (1) (2020) 108227, https://doi.org/10.1016/j.celrep.2020.108227.
[227] M. Forlenza, I.R. Fink, G. Raes, G.F. Wiegertjes, Heterogeneity of macrophage activation in fish, Epub 20110323, Dev. Comp. Immunol. 35 (12) (2011) 1246–1255, https://doi.org/10.1016/j.dci.2011.03.008. PubMed PMID: 21414343.
[228] A.S. Wentzel, J. Petit, W.G. van Veen, I.R. Fink, M.H. Scheer, M.C. Piazzon, et al., Transcriptome sequencing supports a conservation of macrophage polarization in fish, Epub 20200810, Sci. Rep. 10 (1) (2020) 13470, https://doi.org/10.1038/s41598-020-70248-y.
[229] X.J. Lu, Q. Chen, Y.J. Rong, F. Chen, J. Chen, CXCR3.1 and CXCR3.2 differentially contribute to macrophage polarization in teleost fish, Epub 20170512, J. Immunol. 198 (12) (2017) 4692–4706, https://doi.org/10.4049/jimmunol.1700101.
[230] Y. Hu, X. Wei, Z. Liao, Y. Gao, X. Liu, J. Su, et al., Transcriptome analysis provides insights into the markers of resting and LPS-activated macrophages in grass carp (*Ctenopharyngodon idella*), Epub 20181112, Int. J. Mol. Sci. 19 (11) (2018), https://doi.org/10.3390/ijms19113562.
[231] Y. Xie, S. Tolmeijer, J.M. Oskam, T. Tonkens, A.H. Meijer, M.J.M. Schaaf, Glucocorticoids inhibit macrophage differentiation towards a pro-inflammatory phenotype upon wounding without affecting their migration, Epub 20190530, Dis Model Mech 12 (5) (2019), https://doi.org/10.1242/dmm.037887.

[232] S. Skugor, K.A. Glover, F. Nilsen, A. Krasnov, Local and systemic gene expression responses of Atlantic salmon (*Salmo salar* L.) to infection with the salmon louse (*Lepeophtheirus salmonis*), Epub 20081023, BMC Genom. 9 (2008) 498, https://doi.org/10.1186/1471-2164-9-498.
[233] F.R. Hammond, A. Lewis, Z.C. Speirs, H.E. Anderson, T. Sipka, L.G. Williams, et al., An arginase 2 promoter transgenic illuminates immune cell polarisation in zebrafish, Dis Model Mech 16 (2023) 049966, https://doi.org/10.1242/dmm.049966.
[234] A. Lewis, P.M. Elks, Hypoxia induces macrophage tnfa expression via cyclooxygenase and prostaglandin E2 *in vivo*, Epub 20190927, Front. Immunol. 10 (2019) 2321, https://doi.org/10.3389/fimmu.2019.02321.
[235] P.M. Elks, S. Brizee, M. van der Vaart, S.R. Walmsley, F.J. van Eeden, S. A. Renshaw, et al., Hypoxia inducible factor signaling modulates susceptibility to mycobacterial infection via a nitric oxide dependent mechanism, Epub 20131219, PLoS Pathog. 9 (12) (2013) e1003789, https://doi.org/10.1371/journal.ppat.1003789.
[236] H.J. Anders, M. Ryu, Renal microenvironments and macrophage phenotypes determine progression or resolution of renal inflammation and fibrosis, Kidney Int. 80 (9) (2011) 915–925, https://doi.org/10.1038/ki.2011.217.
[237] C.F. Nathan, J.B. Hibbs Jr., Role of nitric oxide synthesis in macrophage antimicrobial activity, Curr. Opin. Immunol. 3 (1) (1991) 65–70, https://doi.org/10.1016/0952-7915(91)90079-G.
[238] R. Bianchini, F. Roth-Walter, A. Ohradanova-Repic, S. Flicker, K. Hufnagl, M. B. Fischer, et al., IgG4 drives M2a macrophages to a regulatory M2b-like phenotype: potential implication in immune tolerance, Epub 20181128, Allergy 74 (3) (2019) 483–494, https://doi.org/10.1111/all.13635.
[239] S.K.E. Harjula, M.J.T. Ojanen, S. Taavitsainen, M. Nykter, M. Rämet, Interleukin 10 mutant zebrafish have an enhanced interferon gamma response and improved survival against a *Mycobacterium marinum* infection, Sci. Rep. 8 (1) (2018) 10360, https://doi.org/10.1038/s41598-018-28511-w.
[240] J.M. Dzik, Evolutionary roots of arginase expression and regulation, Front. Immunol. 5 (2014), https://doi.org/10.3389/fimmu.2014.00544.
[241] F.R. Hammond, Targeting Host-Derived Signalling to Activate Leukocytes and Improve Infection Outcomes in Zebrafish Models, University of Sheffield, 2022. https://etheses.whiterose.ac.uk/31116/.
[242] L. Marjoram, A. Alvers, M.E. Deerhake, J. Bagwell, J. Mankiewicz, J.L. Cocchiaro, et al., Epigenetic control of intestinal barrier function and inflammation in zebrafish, Epub 20150217, Proc. Natl. Acad. Sci. U.S.A. 112 (9) (2015) 2770–2775, https://doi.org/10.1073/pnas.1424089112.
[243] T. Sipka, S.A. Park, R. Ozbilgic, L. Balas, T. Durand, K. Mikula, et al., Macrophages undergo a behavioural switch during wound healing in zebrafish, Free Radic. Biol. Med. 192 (2022) 200–212, https://doi.org/10.1016/j.freeradbiomed.2022.09.021.
[244] N. Takeda, E.L. O'Dea, A. Doedens, J.W. Kim, A. Weidemann, C. Stockmann, et al., Differential activation and antagonistic function of HIF-α isoforms in macrophages are essential for NO homeostasis, Genes Dev. 24 (5) (2010) 491–501, https://doi.org/10.1101/gad.1881410. PubMed PMID: 20194441; PubMed Central PMCID: PMCPMC2827844.
[245] C.A. Madigan, C.J. Cambier, K.M. Kelly-Scumpia, P.O. Scumpia, T.Y. Cheng, J. Zailaa, et al., A macrophage response to *Mycobacterium leprae* phenolic glycolipid initiates nerve damage in leprosy, 85.e10, Cell 170 (5) (2017) 973–985, https://doi.org/10.1016/j.cell.2017.07.030.
[246] J.R. Mathias, M.E. Dodd, K.B. Walters, S.K. Yoo, E.A. Ranheim, A. Huttenlocher, Characterization of zebrafish larval inflammatory macrophages, Dev. Comp. Immunol. 33 (11) (2009) 1212–1217, https://doi.org/10.1016/j.dci.2009.07.003.
[247] J.M. Davis, L. Ramakrishnan, The role of the granuloma in expansion and dissemination of early tuberculous infection, Cell 136 (1) (2009) 37–49, https://doi.org/10.1016/j.cell.2008.11.014.
[248] N. Denans, N.T.T. Tran, M.E. Swall, D.C. Diaz, J. Blanck, T. Piotrowski, An antiinflammatory activation sequence governs macrophage transcriptional dynamics during tissue injury in zebrafish, Nat. Commun. 13 (1) (2022) 5356, https://doi.org/10.1038/s41467-022-33015-3.
[249] E.L. Benard, S.J. Roobol, H.P. Spaink, A.H. Meijer, Phagocytosis of mycobacteria by zebrafish macrophages is dependent on the scavenger receptor Marco, a key control factor of pro-inflammatory signalling, Dev. Comp. Immunol. 47 (2) (2014) 223–233, https://doi.org/10.1016/j.dci.2014.07.022.
[250] E.L. Benard, P.I. Racz, J. Rougeot, A.E. Nezhinsky, F.J. Verbeek, H.P. Spaink, et al., Macrophage-expressed perforins mpeg1 and mpeg1.2 have an anti-bacterial function in zebrafish, Epub 20140919, J. Innate Immun. 7 (2) (2015) 136–152, https://doi.org/10.1159/000366103.
[251] M.R. Cronan, R.W. Beerman, A.F. Rosenberg, J.W. Saelens, M.G. Johnson, S. H. Oehlers, et al., Macrophage epithelial reprogramming underlies mycobacterial granuloma formation and promotes infection, Immunity 45 (4) (2016) 861–876, https://doi.org/10.1016/j.immuni.2016.09.014.
[252] M.R. Cronan, E.J. Hughes, W.J. Brewer, G. Viswanathan, E.G. Hunt, B. Singh, et al., A non-canonical type 2 immune response coordinates tuberculous granuloma formation and epithelialization, Cell 184 (7) (2021) 1757–1774 e14.
[253] J.M. Davis, H. Clay, J.L. Lewis, N. Ghori, P. Herbomel, L. Ramakrishnan, Realtime visualization of mycobacterium-macrophage interactions leading to initiation of granuloma formation in zebrafish embryos, Immunity 17 (6) (2002) 693–702, https://doi.org/10.1016/s1074-7613(02)00475-2.
[254] H. Clay, J.M. Davis, D. Beery, A. Huttenlocher, S.E. Lyons, L. Ramakrishnan, Dichotomous role of the macrophage in early *Mycobacterium marinum* infection of the zebrafish, Cell Host Microbe 2 (1) (2007) 29–39, https://doi.org/10.1016/j.chom.2007.06.004.
[255] F. Sommer, V. Torraca, S.M. Kamel, A. Lombardi, A.H. Meijer, Frontline Science: antagonism between regular and atypical Cxcr3 receptors regulates macrophage migration during infection and injury in zebrafish, J. Leukoc. Biol. 107 (2) (2020) 185–203, https://doi.org/10.1002/jlb.2hi0119-006r. Epub 20190917.
[256] F. Sommer, V. Torraca, Y. Xie, A.E.Int Veld, J. Willemse, A.H. Meijer, Disruption of Cxcr3 chemotactic signaling alters lysosomal function and renders macrophages more microbicidal, Cell Rep. 35 (2) (2021), https://doi.org/10.1016/j.celrep.2021.109000.
[257] F.J. Roca, L.J. Whitworth, S. Redmond, A.A. Jones, L. Ramakrishnan, TNF induces pathogenic programmed macrophage necrosis in tuberculosis through a mitochondrial-lysosomal-endoplasmic reticulum circuit, e11. Epub 20190829, Cell 178 (6) (2019) 1344–1361, https://doi.org/10.1016/j.cell.2019.08.004.
[258] V. Miskolci, J. Squirrell, J. Rindy, W. Vincent, J.D. Sauer, A. Gibson, et al., Distinct inflammatory and wound healing responses to complex caudal fin injuries of larval zebrafish, Elife 8 (2019) e45976, https://doi.org/10.7554/eLife.45976.
[259] T.A. Petrie, N.S. Strand, C.T. Yang, J.S. Rabinowitz, R.T. Moon, Macrophages modulate adult zebrafish tail fin regeneration, Development 141 (13) (2014) 2581–2591, https://doi.org/10.1242/dev.098459.
[260] L.C. Paredes, R.B.D.S. Luz, O.N. Tozzi, L.A.S.J. de Carvalho, S.LdM. Calado, B. N. Padovani, et al., Distinct macrophage phenotypes and redox environment during the fin fold regenerative process in zebrafish, Scand. J. Immunol. 94 (2) (2021) e13026, https://doi.org/10.1111/sji.13026.
[261] M.M. Reimer, I. Sörensen, V. Kuscha, R.E. Frank, C. Liu, C.G. Becker, et al., Motor neuron regeneration in adult zebrafish, J. Neurosci. 28 (34) (2008) 8510–8516, https://doi.org/10.1523/jneurosci.1189-08.2008.
[262] T.M. Tsarouchas, D. Wehner, L. Cavone, T. Munir, M. Keatinge, M. Lambertus, et al., Dynamic control of proinflammatory cytokines Il-1β and Tnf-α by macrophages in zebrafish spinal cord regeneration, Nat. Commun. 9 (1) (2018) 4670, https://doi.org/10.1038/s41467-018-07036-w.
[263] F.A. Bruton, A. Kaveh, K.M. Ross-Stewart, G. Matrone, M.E.M. Oremek, E. G. Solomonidis, et al., Macrophages trigger cardiomyocyte proliferation by increasing epicardial vegfaa expression during larval zebrafish heart regeneration, e5. Epub 20220609, Dev. Cell 57 (12) (2022) 1512–1528, https://doi.org/10.1016/j.devcel.2022.05.014.