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REVIEW ARTICLE

Immune Cell Dynamics in the CNS: Learning From the Zebrafish

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A major question in research on immune responses in the brain is how the timing and nature of these responses influence physiology, pathogenesis or recovery from pathogenic processes. Proper understanding of the immune regulation of the human brain requires a detailed description of the function and activities of the immune cells in the brain. Zebrafish larvae allow long-term, noninvasive imaging inside the brain at high-spatiotemporal resolution using fluorescent transgenic reporters labeling specific cell populations. Together with recent additional technical advances this allows an unprecedented versatility and scope of future studies. Modeling of human physiology and pathology in zebrafish has already yielded relevant insights into cellular dynamics and function that can be translated to the human clinical situation. For instance, *in vivo* studies in the zebrafish have provided new insight into immune cell dynamics in granuloma formation in tuberculosis and the mechanisms involving treatment resistance. In this review, we highlight recent findings and novel tools paving the way for basic neuroimmunology research in the zebrafish.

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Introduction

It is well established that the immune system plays an important role in brain homeostasis and most conditions affecting the brain, including neurodegenerative diseases, psychiatric diseases and neurodevelopmental disorders (Lucin and Wyss-Coray, 2009; Prinz and Priller, 2014; Schwartz et al., 2013). Our current understanding of neuroimmunology, the complex interactions of the immune system with the central nervous system (CNS), however, is limited and many fundamental questions are still unanswered. Basic questions concerning the ontogeny of the brain's immune cells and glia, their functional phenotypes, the life-span of brain immune cells and the effect of aging remain to be answered and are essential for a better understanding of the role of the immune system in CNS health and disease (Streit, 2006; Ginhoux et al., 2013; Biber et al., 2014).

A prerequisite for a more comprehensive description of immunological processes in the brain is a thorough characterization of the function of the different types of immune cells involved. This can be achieved in animal model systems by long-term visualization and mapping of neuroimmune cellular dynamic interactions in the living brain.

In the last decade, the zebrafish has gained substantial popularity as a model for basic research as well as translational biomedical research. Also in neuroscience research, the use of zebrafish as a model is now quickly gaining momentum. Recent technical advances including genome editing (Hwang et al., 2013; Sander et al., 2011; Schmid and Haass, 2013), optogenetics (Teh et al., 2010; Weber and Koster, 2013), fluorescent imaging tools (Giepmans et al., 2006; Mickoleit et al., 2014), and high-throughput behavioral screens have highlighted the use of zebrafish in understanding brain development and function and sped up the discovery of novel psychoactive drugs (Kokel et al., 2010; Rihel et al., 2010).

An important use of zebrafish in biomedical research, is the very powerful possibility for *in vivo* high-resolution imaging of dynamic cellular and even subcellular pathogenic mechanisms in transparent larvae. This has yielded detailed *in vivo* mechanistic insight into developmental and disease processes, including tissue regeneration (Andersson et al., 2012; Zhang et al., 2013), various types of cancer (Chapman et al., 2014; Feng et al., 2010; Langenau et al., 2005; Stoletov

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et al., 2010) and infectious disease (Colucci-Guyon et al., 2011; Davis et al., 2002; Tobin et al., 2012). Although a similar level of in depth genetic, cellular and molecular understanding in neuroimmunology is still far away, this is clearly feasible. Comparable imaging of *in vivo* cell biology at micron-scale level is hard to achieve in the human brain or in classical mammalian models. Clearly, knowledge about these cellular dynamics may be essential to better understand and eventually treat brain diseases.

The main purpose of this review is not as much to give a summary of recent studies but to illustrate the unique application of the zebrafish for detailed long-term monitoring of motile immune cells in the healthy CNS as well as in a disease context. To do so, we will make a case for the specific niche the zebrafish occupies as a discovery platform in the field of neuroimmunology and brain disease research to help resolve *in vivo* mechanisms that are unlikely to be studied in another way. Data derived from these zebrafish studies are in general complementary to studies in other animal models rather than redundant, help to generate hypotheses, provide unexpected novel *in vivo* insights, and sometimes allow for very rapid preclinical development.

First, we will provide background on the conservation of the zebrafish CNS and immune system, relevant to consider the strengths and weaknesses of zebrafish as model for neuroinflammation. Subsequently, we will describe illustrative examples of studies using zebrafish showing how conceptual questions can be addressed yielding unexpected *in vivo* insight in disease-related mechanisms, with sometimes direct clinical relevance. We conclude by consolidating recent technical advances to illustrate what this has yielded so far, and provide several examples already showing the tremendous potential and technical possibilities to address some of the main questions related to functions and origins of immune cells in the brain.

Hereby this review should provide convincing arguments to apply the zebrafish as a tool, allowing an unprecedented view into functions of cellular behavior and contribution to pathogenic mechanisms with the realistic possibility of extrapolating basic findings to patients. Although linear translation of a human CNS disease to zebrafish and back may often not be possible, relevant insights into immune cell dynamics related to such CNS diseases as well as discovering novel concepts that can be translated to more clinical models is very well achievable.

Abbreviations

APC	antigen presenting cell
BBB	blood brain barrier
CNS	central nervous system
$CreERT^2$	Cre recombinase—estrogen receptor T2
EAE	experimental autoimmune encephalitis
FP	fluorescent protein
GFAP	glial fibrillary acidic protein
GFP	green fluorescent protein
Mhc	major histocompatibility complex
NLR	NOD-like receptor
NTR	nitroreductase
SGZ	subgranular zone
SVZ	subventricular zone
TILLING	Targeting induced local lesions in genomes
TLR	Toll-like receptor

The Zebrafish as an *In Vivo* Model for Human Disease

The zebrafish, a small teleost fish native to streams of the south eastern Himalaya, was first introduced as a model organism for developmental biology by George Streisinger in the late 1960s, mainly because of its rapid, completely external embryonic development (Streisinger et al., 1981). Within 24 h after fertilization several organs and cell types, have formed, and are already functioning including the heart, circulation and early innate immune cells capable of ingesting dying cells and bacterial pathogens (Herbomel et al., 1999). Behaviors requiring interactions between different neuronal circuits, such as hunting and capturing prey animals, also develop within the first days of development (Bianco et al., 2011; Budick and O'Malley, 2000; Muto and Kawakami, 2013). Because of their transparency, small size, and rapid development of organs and tissue including the CNS and the immune system zebrafish embryos and larvae have been used extensively for *in vivo* imaging studies of organ development and behavioral research.

The Zebrafish Brain

In vertebrates, including the zebrafish, the embryonic development of the CNS involves formation of the neural tube, which subsequently folds in an intricate manner, creating more distinct fore-, mid-, and hindbrain regions. Eventually, by cell-migration and differentiation these regions further develop into the cerebrum, thalamus and hypothalamus, tectum, tegmentum, cerebellum, pons, and medulla (Blader and Strahle, 2000; Wilson et al., 2002). The main vertebrate cell types in the mammalian CNS, including neurons and glial cells, such as oligodendrocytes, microglia (Fig. 1B,D; Table 1) and astrocytes, have been identified in the zebrafish, as well as specialized barrier structures such as the blood–brain barrier (BBB) and the choroid plexus (Fleming et al., 2013; Garcia-Lecea et al., 2008; Herbomel et al., 2001; Marcus and Easter, 1995; Park et al., 2002; Peri and Nusslein-Volhard, 2008). Main inhibitory as well as excitatory neurotransmitter systems, including GABAergic, glutamatergic, cholinergic, dopaminergic and serotonergic neurotransmission are highly conserved at all levels in the zebrafish (Panula et al., 2010).

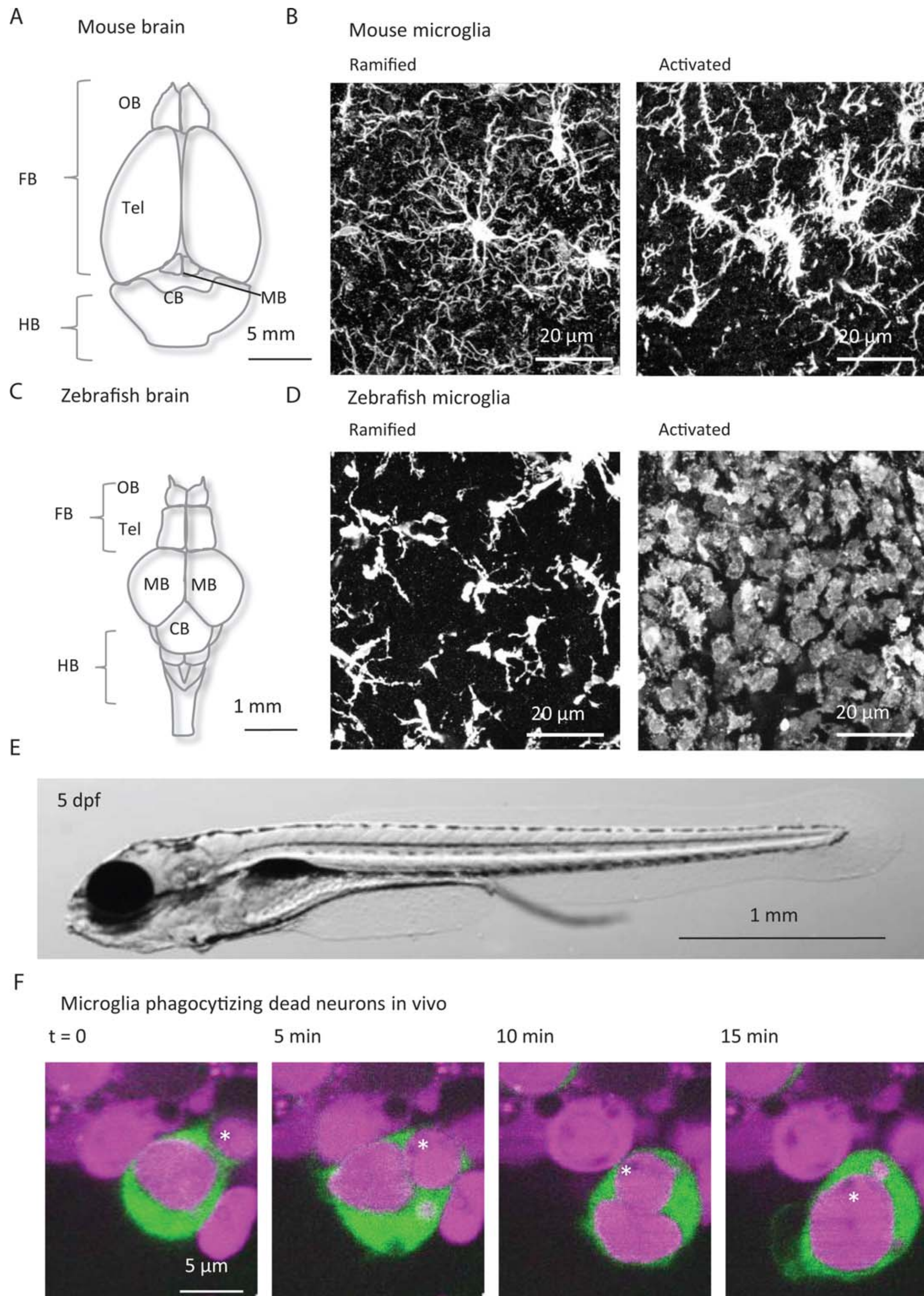

FIGURE 1: Conserved anatomic features of the brain in vertebrates and conserved microglial morphology and behavior. (A), (C) Schematic representations of the mouse (top) and zebrafish brain (bottom). FB = forebrain, OB = olfactory bulb, Tel = telencephalon, MB = midbrain, HB = hindbrain, and CB = cerebellum. (B) Mouse brain sections showing Iba1 antibody-stained microglia in ramified (left) and activated state (right). (D) Zebrafish brain sections showing L-plastin antibody-stained microglia (left) in ramified and activated, amoeboid state (right). (E) 5-day-old zebrafish larva. (F) Stills of confocal microscopic time-lapse recording showing engulfment of an apoptotic neuron (magenta, neuronal red fluorescent protein) by a microglia cell (green, microglial green fluorescent protein) in the brain of a 3-day-old zebrafish larva.

TABLE 1: Conservation of the CNS and immune system between mammals (e.g., human) and teleosts (e.g., zebrafish)

	Mammals	Zebrafish
CNS		
Main structures		
Forebrain (cerebrum, thalamus, hypothalamus)	+	+
Midbrain (tectum, tegmentum)	+	+
Hindbrain (cerebellum, pons, medulla)	+	+
Blood brain barrier	+	+
Meninges	+	+
Choroid plexus	+	+
Ventricular system	+	+
CNS cell types		
Neurons	+	+
Oligodendrocytes	+	+
Astrocytes	+	+/-
Microglia	+	+
Major neurotransmitter systems		
Amino acids (glutamate, GABA, glycine)	+	+
Monoamines (dopamine, serotonin)	+	+
Peptides (somatostatin, opioids)	+	+
Other (acetylcholine)	+	+
Immune system		
Main structures		
Thymus	+	+
Bone marrow	+	-
Lymph nodes & antigen presentation	+	+/-
Lymphatic system	+	+
Leukocytes		
Mononuclear phagocytes (monocytes, macrophage, APCs)	+	+
Granulocytes (neutrophils, eosinophils)	+	+
Lymphocytes (T cells, B cells, NK cells)	+	+/-
Molecular components		
Myeloid differentiation (PU.1, IRF8, CSF1R)	+	+
PRRs (Pattern recognition receptors e.g. TLRs)	+	+
Complement cascade	+	+
Transcription factors (NFκB)	+	+
Antigen presentation (MHC II)	+	+
Cytokine signaling (IL-1β, IL-4, IL-10, IL-6, TGFβ, IFNγ, TNFα)	+	+
Chemokine signaling	+	+

Major structures, cell types and molecular pathways in the CNS and immune system share a high level of conservation between mammals and teleosts (e.g., zebrafish). Some of the differences indicated in this table are in fact not as distinct when viewed from a different perspective. Although zebrafish have no hematopoietic bone marrow, hematopoiesis occurs largely in the kidney marrow, which is at least to a large extent functionally equivalent to the mammalian bone marrow. The same applies to lymph nodes: although lymph nodes are absent in fish, antigen presentation, the main function of lymph nodes, occurs by antigen presenting cells but elsewhere. Although teleosts exhibit a blood brain barrier, the meninges is structurally different from mammals. In mammals the meninges consists of three layers, whereas in teleost only one membranous layer is present known as the primitive meninx. With regard to genetic conservation, it is important to note that a an ancient genome duplication occurred in teleosts. Therefore, it is estimated that 30% of genes have a duplicate variant, that may or may not exhibit redundancy and share the same function. Second, although main immune signaling pathways are highly conserved even across invertebrates, in particular the situation for chemokines is more complex, as they differ quite extensively (Bajoghli, 2013; Nomiyama et al., 2008). In fact even among mammals several chemokines are not conserved at the sequence level, and functional homologs remain to be identified.

Although most basic anatomical regions, cells, and neurotransmitters are conserved between zebrafish and mammals, there are a few important differences (Fig. 1A,B; Tables 1 and 2). For example, fish have nociceptive pathways and respond to noxious stimuli but it is still unclear whether fish can perceive pain, which is considered to be a higher cognitive process (Malafoglia et al., 2013). The neocortex, arguably the most distinguishing feature of the human brain, is thought to be exclusively found in mammals. Fish only have a primitive cerebral brain structure lacking these regions important for higher cognitive functions. However, higher cognitive processes have been described in birds and reptiles suggesting

TABLE 2: Online zebrafish resources

Organization/Resource	Website
Zebrafish Information Network (ZFIN)	ZFIN.org
Zebrafish International resource center (ZIRC)	zirc.org
European zebrafish resource center (EZRC)	ezrc.kit.edu
National BioResource Project Zebrafish	http://www.shigen.nig.ac.jp/zebra/index_en.html
Zebrafish Disease Models Society	www.zdmsociety.org
Zebrafish Mutation Project (Sanger Institute)	sanger.ac.uk/Projects/D_rerio/zmp/
Zebrafish Brain Atlas	zebrafishbrain.org
Zebrafish Atlas	zfatlas.psu.edu/index.php
Zebrafish Genome (Ensembl)	www.ensembl.org/Danio_rerio/Info/Index
Zebrafish Genome (Sanger)	sanger.ac.uk/resources/zebrafish/genoproject.html
Zebrafish Genome (NCBI)	ncbi.nlm.nih.gov/genome?term=danio%20rerio

there likely is functional conservation located elsewhere. Similarly, the dorsal pallium in nonmammalian vertebrates is thought to provide functions equivalent to the mammalian hippocampus, for example with regard to spatial navigation (Kempermann, 2012).

Another major difference is the high abundance of neurogenic zones in the adult zebrafish brain and their high regenerative capacity, which in the adult mammalian CNS is thought to be mainly restricted to the subventricular zone (SVZ) and subgranular zone (SGZ; Grandel and Brand, 2013). One interesting line of thought, suggests that the strong regenerative capacity of the nonmammalian vertebrate CNS may have become repressed in mammals during evolution (Powell et al., 2013). An interesting finding, which supports this idea is a study by Powell and colleagues, who showed correlation between DNA demethylation and regeneration-associated gene expression in a zebrafish model for retinal injury. In particular methylated promoter regions of genes important for regeneration were demethylated in regenerating retina, whereas the same regions in mammals were not (Powell et al., 2013). This suggests that the zebrafish could be suitable for identifying regenerative programs that could possibly be switched on in adult mammals as a potential therapeutic option in traumatic brain injury, brain damage arising from stroke, and other diseases involving loss of neurons.

Zebrafish Immunity

Most major human immune cell lineages, such as macrophages, neutrophils, lymphocytes-B, and T cells, have been identified in the zebrafish (Page et al., 2013; Renshaw and Trede, 2012; Trede et al., 2004). The development of zebrafish immune cells follows a similar differentiation trajectory and is controlled by a similar transcriptional program as found in mammals, involving waves of primitive and definitive hematopoiesis (Ellett and Lieschke, 2010). During primitive hematopoiesis, cells of the intermediate cell mass, the zebrafish equivalent of the primitive blood islands in the mammalian yolk sac, become either myeloid lineage cells or erythroid cells. This decision depends on Pu.1 and Gata1-dependent transcriptional activity, respectively, similar to mammals (Belele et al., 2009; Ellett and Lieschke, 2010). During definitive hematopoiesis, starting 1-day postfertilization, hematopoietic stem cells (HSCs) are formed which expand into erythroid lineage cells and other myeloid lineage cells (Davidson and Zon, 2004; Ellett and Lieschke, 2010).

The adaptive branch of the immune system develops at a later stage which allows a window in which the innate immune system can be studied without involvement of the adaptive system. In zebrafish generation of lymphocytes—lymphopoiesis—starts around 3 days after fertilization (Langenau and Zon, 2005; Willett et al., 1999, 2001). One major difference between the fish and mammalian adaptive immune system is the absence of lymph nodes in fish species in general (Isogai et al., 2009). However, zebrafish do have a repertoire of major histocompatibility complex II (MhcII) expressing antigen presenting cells, B- and T-lymphocytes and a lymphatic system, showing that functional components of the adaptive immune system are present (Langenau and Zon, 2005; Lewis et al., 2014; Renshaw and Trede, 2012; Yaniv et al., 2006). Quintana et al. (2010) demonstrated that the zebrafish has active mechanisms of self-tolerance by showing that the zebrafish ortholog for mammalian Foxp3 (zFoxp3), which is involved in the regulation of self-tolerance,

controlled expression of IL-17, which is associated with autoimmune pathology in mammals. Furthermore, zFoxp3 induced a regulatory phenotype on mouse T-cells, indicating that zFoxp3 has a similar function as mammalian Foxp3 (Quintana et al., 2010). This suggests that the adaptive immune system in the zebrafish has fundamental similarities with mammalian adaptive immunity.

In addition to immune cell types, many of the mammalian immune receptor classes (e.g., TLRs, NLRs), signaling pathways and inflammatory mediators (e.g., interleukins, complement) are conserved in the zebrafish (Hall et al., 2009; Meijer and Spaink, 2011; van der Vaart et al., 2013; Zhang and Cui, 2014; Tables 1 and 2). Furthermore, cellular responses to different immune challenges such as pathogens (Stockhammer et al., 2009; Torraca et al., 2014; Volkman et al., 2010), wounding (Yoo et al., 2011), and cancer (Feng et al., 2012) are similar. With regard to genetic conservation, it is important to note that an ancient genome duplication has occurred in teleosts. Therefore, it is estimated that 30% of genes have a duplicate variant, that may or may not exhibit redundancy and share the same function (Postlethwait et al., 2000). Second, although several main immune signaling pathways are highly conserved even across invertebrates, for example TLRs were discovered in fruit flies, the situation for chemokines in particular is a bit more complicated. Chemokine signaling molecules differ quite extensively between mammals and teleosts (Bajoghli, 2013; Nomiyama et al., 2008). In fact even among mammals several chemokines are not conserved at the sequence level, and many functional homologs remain to be identified in the zebrafish.

Zebrafish CNS Immune Cells

Microglia are the resident immune cells of the CNS. They develop from primitive yolk sac-derived macrophages which colonize the CNS during embryogenesis and differentiate into microglia in a Spi1/Pu.1-transcription factor dependent manner (Herbomel et al., 1999, 2001). Interestingly, this process was identified in the zebrafish about a decade before an analogous Spi1/Pu.1-dependent process, was identified in mammals (Ginhoux et al., 2010; Kierdorf et al., 2013). Similar to mammalian microglia, zebrafish microglia are ramified cells (Fig. 1C,D) with dynamic processes that are scanning their environment. Upon infection or injury they can immediately respond by migrating to the relevant site, and are capable of efficiently phagocytizing bacteria and neuronal debris (Herbomel et al., 1999, 2001; Peri and Nusslein-Volhard, 2008). Pioneering studies by Philippe Herbomel showed that colonization of the CNS by early macrophages, which are microglial precursors, during early development involves a tyrosine kinase receptor named macrophage-colony stimulating factor receptor (CSF1R), a key regulator of the myeloid lineage. In a zebrafish mutant for the Csf1r gene these early macrophages initially fail to reach the CNS, although later colonization of the CNS by microglia appears to take place. Because zebrafish have two homologs of Csf1r, it is possible that the second receptor is sufficient for eventual microglial development (Herbomel et al., 2001). In Csf1r knock out mice microglia fail to develop indicating that Csf1r-dependent microglia development is conserved between fish and mammals (Ginhoux et al., 2010). It is unclear how Csf1r signaling exactly controls microglial development, as this appears to differ from development of other myeloid lineages. Additionally, a recent study shows the requirement for Csf1r signaling in microglial proliferation in mouse disease models (Gomez-Nicola et al., 2013). Although the role of Csf1r in microglial proliferation has not been investigated in adult zebrafish this highlights the potential strength of zebrafish as a model to discover concepts directly relevant to mammalian and disease biology (Gomez-Nicola et al., 2013; Wang et al., 2012).

Astrocytes are highly abundant glial cells in the mammalian CNS, and although they are of ectodermal origin, they have immunological capabilities as well (Gimsa et al., 2013). They are often identified by their high expression levels of the intermediate filament glial fibrillary acidic protein (GFAP). In the zebrafish brain radial glia are the main GFAP-expressing cells (Baumgart et al., 2012; Marcus and Easter, 1995). However, they possess properties attributed to astrocytes as well as radial glia in mammals. Whereas mammalian astrocytes are stellate cells with multiple processes, zebrafish radial glia cell bodies are localized at the ventricle with a single long process spanning the brain, more reminiscent of mammalian radial glia. As well, they share the strong neurogenic potential with mammalian radial glia. Typical properties shared with mammalian astrocytes include glutamate re-uptake from the synaptic cleft by the glutamate transporter Glt-1 (McKeown et al., 2012). Additionally, in an adult zebrafish neuronal injury model radial glia respond in a manner similar to mammalian astrocytes (Baumgart et al., 2012; Goldshmit et al., 2012; Kroehne et al., 2011).

Thus, although zebrafish radial glia show neurogenic potential and morphology characteristic of mammalian radial glia, they also show functional properties of mammalian astrocytes, suggesting they are partly functional equivalents of mammalian radial glia as well as astrocytes.

In Vivo Cell Biology in the Zebrafish

Recent developments in fluorescent imaging technology and the generation of transgenic zebrafish expressing fluorescent proteins labeling specific proteins, organelles and cells of interest make the zebrafish an increasingly powerful model organism (Fig. 1E,F). The development of effective genome editing strategies allows creation of virtually any type of

genetic modification. We will highlight how combinations of these developments have led to basic insight in disease related processes, some of which have turned out to be of direct clinical use. This will illustrate the specific advantages of this model system and the expected benefit of using these models in the context of CNS disease.

Transgenic zebrafish have been very useful for functional genetic research by determining the effect of tissue-specific overexpression of genes of interest. Additionally, transgenic fluorescent reporter lines, labeling specific cell types or tissues using various fluorescent protein (FP) derivatives, is revolutionizing our understanding of *in vivo* cell biology. Initial experiments were directed at expressing green fluorescent protein (GFP)-tagged proteins to label a particular cell type, including different immune cell types allowing live imaging of their function *in vivo* (Long et al., 1997; Meng et al., 1997; Moss et al., 1996). For example, myeloperoxidase driven GFP (mpx-GFP) labels neutrophils, macrophage-expressed gene 1 (mpeg1)-driven GFP labels all mononuclear phagocytes including microglia, whereas apolipoprotein E (ApoE) more specifically labels microglia (Ellett et al., 2011; Peri and Nusslein-Volhard, 2008; Wittamer et al., 2011).

To target transgenes to specific cell types one can search and optimize a minimal promoter region yielding potent expression in the cell type of choice. Such promoters often lack all up and downstream regulatory elements to achieve a faithful representation of the actual expression pattern of the gene or cell type of choice. Other approaches have been developed making it feasible to increase the reporter repertoire substantially. BAC-recombineering takes advantage of recombination in bacteria to insert a sequence of choice into a BAC-clone. By targeting an FP directly behind the ATG translational start site of the gene of choice in a BAC containing the promoter but also more distant regulatory sites, the physiological expression of this gene can be captured (Bussmann and Schulte-Merker, 2011). Hereby the need for initial identification of the required promoter region of the gene is circumvented. Additionally BAC-recombineering allows relatively straightforward creation of multiple reporter genes rapidly. Therefore, this would facilitate the creation of new lineage or activation-state specific reporter lines, for example for newly identified microglial specific genes for which minimal essential promoter regions have not yet been identified (Wieghofer et al., 2015).

Nowadays many types of FPs are available with diverse spectral properties, and localization signals, allowing quantitative *in vivo* visualization of organelles, subcellular processes including ionic fluctuations, activity of signal transduction pathways and macromolecular structural alterations (Akerboom et al., 2013; Hocking et al., 2013; Shaner et al., 2005). For example genetically encoded calcium indicators (GECIs), such as Gcamp, have been optimized and used to study neuronal circuits in the zebrafish (Akerboom et al., 2013; Chen et al., 2013; Muto et al., 2011). Recently, Hochbaum et al. (2014) have developed voltage indicators, which can be used for optical electrophysiological studies. In addition to "normal" FPs, there also is a repertoire of photoconvertible proteins available that allow switching colors of FPs from green to red (e.g., kaede, dendra) or from a dark state to an active fluorescent state (photoactivatable GFP, PA-GFP; Adam et al., 2014; Ando et al., 2002). This allows for spatiotemporal lineage tracing, which will be discussed later. Zebrafish with several of these transgenic markers can be crossbred relatively easily. This yields a virtually endless combination of transgenic reporters. By using combinations of nuclear-, membrane-, and cytoplasmic FPs, with only three colors, 9 different cell types could in principle be labeled at the same time, and imaged at high spatiotemporal resolution in the living brain (Bussmann and Schulte-Merker, 2011; Suster et al., 2009). Alternatively, several subcellular processes could be imaged in the same transgenic fish. In all, the development of FPs with diverse properties allows spatiotemporal labeling *in vivo* at the structural, cellular, subcellular and functional level.

Substantial knowledge on human neuropathology has come from immunohistochemistry using formaldehyde-fixed, paraffin embedded tissue stained for hematoxylin/eosin (H&E) or antibodies thus marking disease specific hallmarks. In contrast, what we learn about *in vivo* mechanisms in zebrafish is generally based on monitoring of cell-specific expression of fluorescent proteins. Using live imaging one can monitor events as they occur *in vivo*. It is sometimes unclear what these features would look like in fixed tissue, preventing direct comparisons and extrapolation of possibly relevant findings *in vivo*. Thus biological events that are rarely found in a specific snapshot in fixed tissue, are more easily identified by long-term video microscopy (Hosseini et al., 2014; van Ham et al., 2014). Additionally, by correlated microscopy imaging live events and static images can be combined thus allowing the extrapolation of biological processes to conventional histopathology (Sjollema et al., 2012). This makes it easier to address more basic fundamental questions in *in vivo* models and translate new meaningful hypotheses to disease mechanisms.

In vivo studies can yield unexpected insight and discoveries that would likely be missed in studies using immunohistochemistry or other techniques that do not provide information about dynamic processes. We will highlight insights and discoveries related to immune cell development and function, to indicate the type of mechanistic insight that can be derived. Second, we will highlight how zebrafish models can lead to clinically applicable concepts and potential drugs for translational medicine approaches. As mentioned in

the introduction, the zebrafish has proven its value as a model in various aspects of neuroscience, however, these are beyond the scope of this review. Examples of these aspects include modeling behavior and complex behavioral brain disorders in adult zebrafish, optogenetics, understanding neurogenesis and neuroregeneration, as well as high throughput behavioral and neuroactive drug discovery (Baraban, 2007; Baraban et al., 2013; Chapouton et al., 2007; Kalueff et al., 2014b; Kokel et al., 2012; Kyritsis et al., 2014).

New Insight in Infection and Immunity From In Vivo Studies in Zebrafish

In vivo studies in the zebrafish have resulted in new, unexpected insights into basic immune responses. For instance, a zebrafish tuberculosis (TBC) infection model has allowed visualization of basic disease mechanisms *in vivo* with regard to pathogen propagation, treatment resistance and genetic vulnerability (Adams et al., 2011; Davis and Ramakrishnan, 2009; Volkman et al., 2010). A hallmark of TBC is the formation of granulomas, which were generally thought of as static structures formed as a protective mechanism by the host organism. Zebrafish studies, however, revealed that these granulomas are highly dynamic structures used by the bacteria for propagation, potentially changing the putative disease mechanism (Davis and Ramakrishnan, 2009). Another example shows very basic immune cell behavior that has been previously unrecognized, and is currently validated in mammalian disease models. In a zebrafish wounding model using transection of the tail fin, resolution of inflammation was accompanied by reverse migration of neutrophils away from the site of injury (Elks et al., 2011; Mathias et al., 2006). Since then, this phenomenon has been shown in mammalian model systems and may be relevant for human disease biology (Buckley et al., 2006; Woodfin et al., 2011). These examples, although they partly remain to be investigated in mammals, indicate the type of unexpected insights found by unbiased *in vivo* imaging experiments that could alter the view on the roles of behavior of immune cells in disease.

A recent elegant example in which *in vivo* imaging was used to elucidate a complex multi-organ feedback loop driving the increased production of granulocytes in response to a cerebral infection was is the study by Hall et al. (2012). By genetic and pharmacological manipulation, and live imaging they showed that in zebrafish larval brains infected with Salmonella bacteria, macrophages secrete granulocyte colony stimulating factor (Gcsf) into the blood. Subsequently they showed that Gcsf receptor activation in the hematopoietic tissue induces expression of the transcription factor C/ebpβ, which then drives expression of nitric oxidase Nos2a, controlling proliferation of HSCs required for the differentiation of new granulocytes (Hall et al., 2012). Although these effects were known already as separate processes, the use of *in vivo* imaging in this zebrafish experiment allowed for the connection between these separate processes, thereby elucidating a complex multi-tissue signaling mechanism in a single study.

Several immune deficiencies with defects in specific hematopoietic lineages have been modeled using zebrafish mutant for homologs of human disease genes. By imaging leukocyte behavior in these mutants better understanding of the functional effect of these mutations on leukocyte behavior has been achieved (Deng et al., 2011; Jones et al., 2013; Walters et al., 2010). Good examples have been provided for Wiskott-Aldrich syndrome (WAS) and X-linked neutropenia (XLN), which are immunodeficiencies caused by mutations in the WASp gene resulting in defective control of the small GTPase Cdc42. Depending on the mutation, this can lead to diverse neutrophil phenotypes ranging from defective generation of neutrophils up to altered chemotaxis and phagocytic capacity. By expression of human disease variants of WASp in a zebrafish background lacking functional WASp, Jones et al. (2013) observed indeed differential effects on leukocyte functioning, the capacity and generation of neutrophils, chemotactic responses to wounding, and the phagocytic capacity of macrophages. These studies thus allow testing of clinically relevant mutations and variants of known immunodeficiencies, to gain a detailed *in vivo* understanding of defects in leukocyte 3-dimensional migratory behavior that may not be apparent in tissue culture models.

Novel Insights in Microglial Phagocytic Responses

Phagocytosis is a key function of immune cells in health and disease. Many brain diseases involve extensive cell death, but how dying neurons are cleared from the CNS has proven hard to visualize, partly because the process is thought to be very efficient. Many components of the engulfment machinery, which are involved in finding, recognizing and engulfing dying cells, and in the subsequent processing of ingested material, have been identified, but how this machinery precisely operates within the complex brain tissue is poorly understood *in vivo* (Nagata et al., 2010; Ravichandran and Lorenz, 2007; Reddien and Horvitz, 2004).

In order to study genes involved in control of engulfment in the brain *in vivo*, we developed a transgenic zebrafish reporter line to label apoptotic cells (van Ham et al., 2010). Fluorescently tagged annexin V (A5) protein, is widely used to detect cell death, by binding with high affinity to phosphatidylserine (PS), which is exposed on the plasma membrane in apoptotic cells (Reutelingsperger and van Heerde, 1997). We created a secreted version of A5 (secA5), to achieve labeling of PS exposure in dying cells *in vivo* (van Ham et al., 2010). Subsequently, by intravital microscopy in secA5-

transgenic zebrafish we visualized how dying neurons are engulfed by microglia in living brains (van Ham et al., 2012). Additionally, by visualizing defective engulfment *in vivo*, we show that the guanine nucleotide exchange factor Elmo1 (Engulfment and Cell Motility protein 1) is needed for completion of engulfment after recognition of the dying cell, via regulation of actinomyosin dynamics in the formation of the phagocytic cup (van Ham et al., 2012). Around the same time Elmo1 was shown to play a role in phagocytic processes required for adult neurogenesis in mice, suggesting this gene may indeed play a role in engulfment under physiological conditions (Lu et al., 2011). More recently, Mazaheri et al. (2014) have used transgenic secA5 expression in live imaging studies as well to visualize PS exposure and clearance of dying neurons. In their study they use secA5 labeling of dying neurons to study the genes bai1 and tim4, previously implicated *in vitro* in recognition and adhesion of apoptotic cells (Kobayashi et al., 2007; Miyanishi et al., 2007; Park et al., 2007). They show that the *in vivo* functions of these two genes, is actually different from their function *in vitro*, as bai1 and tim4 control phagosome formation around dying neurons and stabilization of this phagosome. Together these studies clearly show how *in vivo* imaging data can assist in achieving a molecular level of understanding *in vivo* mechanisms involved in engulfment. It will be interesting to determine how the role of these genes extends to mammals. Second, these studies illustrate that the development of new fluorescent reporters can directly facilitate research into cellular and even subcellular processes by gaining truly unprecedented *in vivo* resolution. Furthermore, unexpected behavior of immune cells provides instructive functional hypotheses for future experiments to test the relevance of phagocytosis behavior in mammalian models.

Before phagocytic cells can perform their task they need to migrate toward the area where they are needed, attracted by chemokines and other cues, and it is critical to understand how and what signaling pathways govern recruitment of these immune cells to specific brain areas. An initial clue of the intercellular signaling mechanisms involved in recruiting microglia to injury comes from a live imaging study in zebrafish by Sieger and coworkers. In a laser mediated neuronal injury model neurons show glutamate receptor (NMDAR) activation leading to Ca^{2+} influx, which causes ATP-dependent microglial migration through purinergic P2Y12 activation (Sieger et al., 2012). Similar observations have been made for mammalian microglia recently. Eyo et al. (2014) showed that during kainate-induced seizures in mouse brain slices and *in vivo*, glutamate-induced microglial responses involve NMDA receptor-dependent Ca^{2+}-influx, followed by ATP release and activation of microglial P2Y12. Interestingly, the same issue of the journal contained a second study that demonstrated that NMDAR activity results in ATP-dependent mouse microglial responses (Dissing-Olesen et al., 2014). These examples clearly show that fundamental biological functions and processes regarding microglia in the CNS are highly conserved. In addition to these studies on microglia responses to wounding, Li et al. (2012) used *in vivo* imaging to show that microglia are induced to contact highly active neurons and decrease their spontaneous firing. Again, these type of conceptual findings may pave the way for more focused studies in mice.

Studying Immune Responses in Zebrafish Models for CNS Disease

Several studies have shown that zebrafish can be used to study CNS disorders, such as neurodegenerative diseases and epilepsy. For example Paquet and colleagues generated a zebrafish model for frontotemporal dementia (FTD) and Alzheimer's disease (AD) by transgenic overexpression of mutant human tau resulting in cell death and disease related tau-phosphorylation (Paquet et al., 2009). Polyglutamine inclusions associated with Huntington's disease have also been induced in the zebrafish (Schiffer et al., 2007). More recently loss of function of Tdp-43, involved in amyotropic lateral sclerosis and FTD, was shown to result in motor neuron axonopathy in the zebrafish (Schmid et al., 2013). Additionally, the zebrafish has been used successfully in studies on other brain conditions including genetically inherited epilepsy known as Dravet syndrome, psychiatric disorders and neurodevelopmental disorders (Kalueff et al., 2014a; Norton, 2013). Immune cell behavior was not the aim of any of these studies, but it would be interesting to compare in these models how immune cells respond to the different disease contexts.

Recently, we have used nitroreductase (NTR)-mediated ablation to specifically induce neuronal cell death in the zebrafish brain (van Ham et al., 2014, 2012). Nitroreductase is a bacterial enzyme known as nsfB, which can convert the antibiotic metronidazole into a DNA crosslinking agent, causing programmed cell death. Transgenic expression of nsfB/NTR has been successfully used in zebrafish to study regeneration after ablation of pancreas, heart and other tissues (Curado et al., 2007; Pisharath et al., 2007). By applying brain-specific expression of nitroreductase we showed that macrophages and microglia, clear dead neurons at the earliest stages after induction of neuronal cell death whereas only microglia dominate at later stages. Interestingly, animals recover from damage completely after ablation at larval stages, even after multiple ablations. The recovery phase involves programmed cell death of phagocytic immune cells, which are cleared by microglia. This is reminiscent of immune cell behavior upon wounding, where immune cells also undergo

programmed cell death in a process known as resolution of inflammation (Serhan and Savill, 2005). We also found that all phagocytes that have engulfed neuronal debris are positive for a marker that labels microglia as well as macrophages, whereas only a subset of phagocytes showed activity of the microglia-specific ApoE-promoter (van Ham et al., 2014). This indicates that peripheral macrophages and resident microglia are recruited to brain injury in zebrafish, and reveals that these two cell types have different roles during their response to neuronal cell death. Another study used nitroreductase (NTR)-mediated ablation in sensory peripheral neurons and showed recovery after ablation in these neurons, which seems to require the presence of peripheral glial cells (Pope and Voigt, 2014). This suggests that depending on the location of neuronal cell death, different cell types are involved in recovery and that NTR ablation can be used to understand immune maintenance of different types of neuronal tissue.

Several other ways of inducing transgenic targeted cell death include expression of the fluorescent protein KillerRed, which upon irradiation with green light can induce oxidative stress-mediated cell death (Teh et al., 2010), and thymidine kinase, which can convert ganciclovir into a cytotoxic agent, killing proliferative cells in particular (Lalancette-Hebert et al., 2007). Such techniques can be used to damage tissues in different ways than NTR, to test if different causes and types of cell death yield the same immunological outcome. Alternatively, these techniques can also be used to ablate specific immune cell lineages, to identify their corresponding functions (Petrie et al., 2014).

Many disease and injury model studies in zebrafish focus on embryos and larvae because of their transparency and small size amongst others. Adult zebrafish models have been developed as well, applying various types of invasive injury including a telencephalic stabwound, spinal cord lesions, and injection of excitotoxic agents (Alfaro et al., 2011; Kroehne et al., 2011; Munzel et al., 2014; Skaggs et al., 2014; Vajn et al., 2014). Alternatively, these paradigms can often be applied to larvae as well. Adult zebrafish are particularly useful to study adult neuroregeneration and may allow assessing whether findings in larval brains apply to adult brain as well, which may better predict their effect in adult mammals. One study using the stab wound paradigm has shown that recovery from a brain stab wound requires an inflammatory response for repair and regeneration to occur (Kyritsis et al., 2012).

Future Perspectives

In view of the recent technological developments the studies mentioned reflect only the onset of the possibilities of the zebrafish as a model for CNS disease. The concept of a genetic model organism is that genes can be manipulated efficiently to study the function of genes for example those involved in immune function. A considerable limitation of application of zebrafish genetics has been the difficulty to generate targeted or conditional knockouts. One useful possibility is the use of TILLING, targeted induced local lesions in genomes, in which ENU mutated fish are screened for mutations in target genes to obtain mutants (Amsterdam and Hopkins, 2006; Wienholds et al., 2003). Recently developed genome editing techniques have made the generation of targeted mutations in the zebrafish much easier. The first of these techniques has been published in 2008 and involves the use of zinc finger proteins (Doyon et al., 2008), followed by recent addition of TALENs and the CRISPR/CAS system (Huang et al., 2014; Hwang et al., 2013; Schmid and Haass, 2013). All these techniques work via the introduction of a double strand break at a specific position in the genome. When targeted at the right position this can cause nonsense mutations or truncated transcripts, leading to disruption of gene function. Although many articles published on this topic in relation to the zebrafish are in fact proof of concept articles, these techniques will be extensively used in the zebrafish to produce a wide variety of mutants. In addition to directly disrupting gene function, these techniques also allow targeted insertion of DNA elements into the genome (Auer and Del Bene, 2014; Auer et al., 2014). Hereby, knock-in animals can be generated, expressing for example FPs from a targeted locus, and the generation of conditional mutants should be possible in combination with the Cre-lox system mentioned below. Thus, these developments have established a versatile genetic toolbox well matched to available approaches in mouse genetics.

Although these new reverse genetics techniques allow creation of mutants, the strength of unbiased forward genetic screening is still appealing. The availability of the sequence of the complete zebrafish genome, the reduced costs and the incredible throughput of massively parallel sequencing approaches greatly facilitates forward screening efforts. An illustrative example of how an ENU mutagenic screen in zebrafish can yield insight in concepts of microglial activity comes from a recent effort to discover new genes essential for microglial function. Shiau and colleagues described that one of the identified mutants showed a complete lack of microglia in the CNS caused by loss of function of the NOD-like receptor nlrc3-like gene (Shiau et al., 2013). NOD-like receptors (NLRs) are cytoplasmic pattern recognition receptors, with important regulatory function in immune cell activity (Saxena and Yeretssian, 2014). Using genetics, live imaging and vital dyes the Shiau et al. (2013) showed that nlrc3-like normally prevents inflammatory activation in microglia during their journey to the brain. Without functional nlrc3-like,

the microglial precursors show an inflammatory, distracted, phenotype preventing them from reaching the brain. Interestingly, the authors did find neutrophils in the brain, consistent with systemic inflammation (Shiau et al., 2013). One concept derived from recent studies on microglia is that they are under strong inhibitory control, which when lost, can lead to toxicity of microglia toward neurons (Biber et al., 2007; Cardona et al., 2006; Ransohoff and Perry, 2009). The study on nlrc3-like very elegantly, although indirectly, shows the concept of a need for inflammatory suppression of immune cells. The homolog of nlrc3-like has not been identified in mammals, but the protein is very similar to human Nlrc3, and it will be interesting to determine the function of mammalian Nlrc3 and other NLRs in microglia. In fact, recent studies linked activation of another NLR, NLRP3, in microglia by amyloid-beta to development of AD (Heneka et al., 2013).

A key issue in neuroimmune research concerns the individual contributions and roles of different types of immune cells in CNS disease. Fate mapping has been used extensively by embryologists to track distribution of embryonic tissues in older animals by for example dye injections, which allowed important insights in developmental biology. Nowadays, single-cell fate mapping techniques, known as lineage tracing, allow detailed single cell insight into cellular origins and distribution which can be combined with intravital imaging. For example Cre recombinase-mediated lox recombination is generally used in mice to generate tissue specific- or conditional genetic knockouts (Sauer, 1998). The bacteriophage gene Cre is expressed in a specific cell type, where it can excise a genetic element based on two flanking genetic elements known as loxP sites. When using Cre, fused to an estrogen receptor fragment (ERT2) the activity of Cre can be induced via treatment with tamoxifen to temporally control Cre activity. In combination with fluorescent proteins CreERT2 can be used to permanently switch on or off expression of a specific fluorescent protein in a given tissue allowing creation of multicolor lineage tracing, as was used in the brainbow mice and zbow zebrafish (Livet et al., 2007; Mosimann et al., 2011). In addition to fluorescent protein expression, this technique can be used to switch genes on or off in cell type specific manner, allowing the creation of conditional knockouts in zebrafish. An example of how this can be used in the context of neuronal injury is shown in a recent study by Kroehne et al. (2011). They showed in adult zebrafish that radial glial cells contribute to new neuronal tissue in response to a telencephalic stab wound by using a radial glial expressed CreERT2. In addition to tracking radial glia cells, such a system could as well be used in the zebrafish to study the origin and fate of immune cells upon damage in the brain. This would allow us to address questions related to the individual contributions of separate populations of macrophages and microglial cells.

Additional questions that can be studied using these approaches for example in combination with an ablation model are related to where specific immune cells come from and where they eventually go to. Is there local or more distant recruitment of microglia, or is there recruitment of peripheral immune cells? Our recent study using *in vivo* and electron microscopy suggests that peripherally derived macrophages can enter or exit the brain via the olfactory nerve in the nasal cavity (van Ham et al., 2014). Although this route has been shown in histological sections in mouse models (Kaminski et al., 2012), it is virtually impossible to capture using live imaging in mice. Other possible routes, which can be easily imaged, include the zebrafish choroid plexus which is formed within 6 days after fertilization (Garcia-Lecea et al., 2008) and the BBB, which is formed within the first few days after fertilization (Fleming et al., 2013). By using lineage tracing techniques, these questions can be conclusively addressed *in vivo*.

Recently, many comprehensive studies have been published showing transcriptome analyses of microglia in mouse models for neurological diseases using microarrays and RNA sequencing (Butovsky et al., 2014; Chiu et al., 2013; Hickman et al., 2013; Orre et al., 2014). Additionally, genetic studies in humans have identified tremendous numbers of potential disease genes and variants. For both these type of data sets zebrafish provides a great platform to analyze expression patterns and test the effect of manipulating genes on immune function or disease progression before moving to mammalian systems or identifying them as causative variants in disease. For example, *in situ* mRNA hybridization of candidate genes in zebrafish is a relatively straightforward way to identify relevant expression patterns. Similarly, BAC recombineering can be used as an *in vivo* alternative to *in situ* RNA detection to identify dynamic expression patterns (Bussmann and Schulte-Merker, 2011). In fact BAC recombineering was applied to create a reporter for expression of P2Y12 marking microglia in zebrafish (Sieger et al., 2012). This gene was later found as a highly enriched microglial gene in several mouse transcriptome studies mentioned (Beutner et al., 2013; Butovsky et al., 2014; Chiu et al., 2013; Hickman et al., 2013). Subsequently mutants can be created for such a gene, and virtually any combination of the techniques mentioned, including tissue-specific conditional markers or loss of function. Taken together with technical advances mentioned above, this suggests that also for genes found in brain disease models by large scale genetic approaches, functional analysis in zebrafish is becoming a useful starting place for functional genomics and to determine the function of these genes.

Small Molecule Screening and Drug Discovery

Another important aspect of zebrafish is their suitability for high-throughput drug screening. Pioneering studies by

Peterson and colleagues in 2001 showed in zebrafish embryos that small molecules, efficiently absorbed from the surrounding liquid, can induce very specific developmental phenotypes (Peterson et al., 2000). Because of their small size, zebrafish embryos and larvae can be kept in 96-well plates. This, in combination with the easiness of treatment with chemical compounds (addition of the compound to the medium) makes them highly suitable for high-throughput drug screens, of which some have already led to direct clinical applications. The prostaglandin derivative dmPGE2, initially identified in zebrafish as a drug that increases the number of HSCs has been approved for phase I clinical trials to enhance engraftment of HSC transplantation in leukemia patients (Goessling et al., 2011; North et al., 2007). Another compound, lenaldekar (LDK), identified in a zebrafish small molecule screen for selective elimination of immature T cells, may be a new drug for treatment of T cell leukemia (Ridges et al., 2012). Interestingly, because multiple sclerosis involves an over-activation of T cells as well, they tested this new drug in an MS mouse model (EAE) showing a reduced disease severity due to prevention of expansion of the T-cell population (Cusick et al., 2012). This also shows that although there are no zebrafish models for autoimmune diseases, yet, zebrafish studies can have direct relevance for research on autoimmunity. A recent example of a novel drug found in zebrafish and moving toward clinical application is based on the drug dorsomorphin, which causes dorsalization in zebrafish embryos and inhibits BMP signaling. Because a fatal connective tissue disease fibrodysplasia ossificans progressiva (FOP) is caused by increased BMP signaling, inhibiting this pathway could inhibit the disease process. The effectivity of the dorsomorphin derivative in a mouse model for this disease has led medicinal chemists to develop more potent derivatives of dorsomorphin that may soon be tested in early clinical trials (Yu et al., 2008a, 2008b). These data clearly indicate that small molecule screens in zebrafish lead to discovery of drugs with high clinical relevance without a priori knowledge of what drug target would be involved. Beyond these screens of disease related phenotypes, and interesting in the context of this review, high-throughput behavioral drug discovery has revealed already many new neuro-psychoactive compounds (Kokel et al., 2010, 2012). Although the exact mechanisms of some of the drugs found are unknown, finding new drug classes is an Achilles heel in drug discovery, as most of the currently known drugs were found serendipitously (Schlueter and Peterson, 2009).

In the context of this review, small molecule screening could be applied to transgenic fluorescent reporter lines marking brain immune cells to find small molecules that alter intensity or localization of these reporters *in vivo*. Two recent examples of screens directed at finding drugs that alter immune cell behavior show that such an approach is feasible. In the first study they identified small molecules that inhibit retrograde migration of neutrophils in a zebrafish tailfin wounding model (Robertson et al., 2014). Interestingly, drugs found in this screen had the same effect on human neutrophils suggesting these responses are highly conserved. In a second study they identified small molecules that inhibits inflammatory recruitment of immune cells to killing of lateral line neuromast cells by copper sulfate (d'Alencon et al., 2010; Wittmann et al., 2012). Imaging fluorescent phenotypes in the brain in 3D has been difficult in a high throughput fashion, mainly due to out of focus light, and the general low throughput nature of confocal microscopy. Recent developments have also started to overcome these hurdles by designing 96 wells plates to mount larvae in the right position for imaging, building microfluidics systems for automated confocal imaging and automated quantitative analysis of the imaging data (Pardo-Martin et al., 2010; Peravali et al., 2011). Therefore, in addition to using the models described throughout this chapter for annotation of gene function, and elucidation of disease mechanisms, the possibility of small molecule screening in zebrafish has become a mainstay in novel bioactive small molecule discovery (Zon and Peterson, 2005).

Concluding Remarks

A recent zebrafish review has suggested that zebrafish models should prove themselves by discovering new biology instead of merely modeling disease processes (Lieschke and Currie, 2007). Based on progress described above this step has clearly been taken. Although many aspects of zebrafish biology with regard to neuroimmunology are highly conserved, obviously, there are differences between the human or mammalian and fish immune system. For example zebrafish do not have lymph nodes. In contrast, zebrafish do have MhcII expressing antigen presenting cells suggesting they have functional equivalents of lymph nodes elsewhere. Also, hardly any of the antibodies used in humans and mice as markers for different immune cell types and inflammatory phenotypes are compatible with the zebrafish. Fortunately, transgenic markers are as versatile as antibodies and can be used for the same purposes equally well. Therefore, for basic questions about the functioning of the immune system such minor differences in mechanism or approach do not have to be limiting. We argue that recent unexpected discoveries and technical advances create a niche for the zebrafish in the field of neuroimmunology by allowing an unprecedented view into cellular mechanisms of immune-maintenance and repair in the brain with the realistic possibility of eventually extrapolating basic findings to the clinic.

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References

Adam V, Berardozzi R, Byrdin M, Bourgeois D. 2014. Phototransformable fluorescent proteins: Future challenges. Curr Opin Chem Biol 20:92–102.

Adams KN, Takaki K, Connolly LE, Wiedenhoft H, Winglee K, Humbert O, Edelstein PH, Cosma CL, Ramakrishnan L. 2011. Drug tolerance in replicating mycobacteria mediated by a macrophage-induced efflux mechanism. Cell 145:39–53.

Akerboom J, Carreras Calderon N, Tian L, Wabnig S, Prigge M, Tolo J, Gordus A, Orger MB, Severi KE, Macklin JJ, Patel R, Pulver SR, Wardill TJ, Fischer E, Schüler C, Chen TW, Sarkisyan KS, Marvin JS, Bargmann CI, Kim DS, Kügler S, Lagnado L, Hegemann P, Gottschalk A, Schreiter ER, Looger LL. 2013. Genetically encoded calcium indicators for multi-color neural activity imaging and combination with optogenetics. Front Mol Neurosci 6:2.

Alfaro JM, Ripoll-Gomez J, Burgos JS. 2011. Kainate administered to adult zebrafish causes seizures similar to those in rodent models. Eur J Neurosci 33:1252–1255.

Amsterdam A, Hopkins N. 2006. Mutagenesis strategies in zebrafish for identifying genes involved in development and disease. Trends Genet 22:473–478.

Andersson O, Adams BA, Yoo D, Ellis GC, Gut P, Anderson RM, German MS, Stainier DY. 2012. Adenosine signaling promotes regeneration of pancreatic beta cells in vivo. Cell Metab 15:885–894.

Ando R, Hama H, Yamamoto-Hino M, Mizuno H, Miyawaki A. 2002. An optical marker based on the UV-induced green-to-red photoconversion of a fluorescent protein. Proc Natl Acad Sci USA 99:12651–12656.

Auer TO, Del Bene F. 2014. CRISPR/Cas9 and TALEN-mediated knock-in approaches in zebrafish. Methods 69:142–150.

Auer TO, Duroure K, De Cian A, Concordet JP, Del Bene F. 2014. Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair. Genome Res 24:142–153.

Bajoghli B. 2013. Evolution and function of chemokine receptors in the immune system of lower vertebrates. Eur J Immunol 43:1686–1692.

Baraban SC. 2007. Emerging epilepsy models: Insights from mice, flies, worms and fish. Curr Opin Neurol 20:164–168.

Baraban SC, Dinday MT, Hortopan GA. 2013. Drug screening in Scn1a zebrafish mutant identifies clemizole as a potential Dravet syndrome treatment. Nat Commun 4:2410.

Baumgart EV, Barbosa JS, Bally-Cuif L, Gotz M, Ninkovic J. 2012. Stab wound injury of the zebrafish telencephalon: A model for comparative analysis of reactive gliosis. Glia 60:343–357.

Belele CL, English MA, Chahal J, Burnetti A, Finckbeiner SM, Gibney G, Kirby M, Sood R, Liu PP. 2009. Differential requirement for Gata1 DNA binding and transactivation between primitive and definitive stages of hematopoiesis in zebrafish. Blood 114:5162–5172.

Beutner C, Linnartz-Gerlach B, Schmidt SV, Beyer M, Mallmann MR, Staratschek-Jox A, Schultze JL, Neumann H. 2013. Unique transcriptome signature of mouse microglia. Glia 61:1429–1442.

Bianco IH, Kampff AR, Engert F. 2011. Prey capture behavior evoked by simple visual stimuli in larval zebrafish. Front Syst Neurosci 5:101.

Biber K, Neumann H, Inoue K, Boddeke HW. 2007. Neuronal 'On' and 'Off' signals control microglia. Trends Neurosci 30:596–602.

Biber K, Owens T, Boddeke E. 2014. What is microglia neurotoxicity (Not)? Glia 62:841–854.

Blader P, Strahle U. 2000. Zebrafish developmental genetics and central nervous system development. Hum Mol Genet 9:945–951.

Bradford Y, Conlin T, Dunn N, Fashena D, Frazer K, Howe DG, Knight J, Mani P, Martin R, Moxon SAT, Paddock H, Pich C, Ramachandran S, Ruef BJ, Ruzicka L, Bauer Schaper H, Schaper K, Shao X, Singer A, Sprague J, Sprunger B, Van Slyke C, Westerfield M. 2011. ZFIN: Enhancements and updates to the zebrafish model organism database. Nucleic Acids Res 39: D822–D829.

Buckley CD, Ross EA, McGettrick HM, Osborne CE, Haworth O, Schmutz C, Stone PC, Salmon M, Matharu NM, Vohra RK, Nash GB, Rainger GE. 2006. Identification of a phenotypically and functionally distinct population of long-lived neutrophils in a model of reverse endothelial migration. J Leukoc Biol 79:303–311.

Budick SA, O'Malley DM. 2000. Locomotor repertoire of the larval zebrafish: Swimming, turning and prey capture. J Exp Biol 203:2565–2579.

Bussmann J, Schulte-Merker S. 2011. Rapid BAC selection for tol2-mediated transgenesis in zebrafish. Development 138:4327–4332.

Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, Koeglsperger T, Dake B, Wu PM, Doykan CE, Fanek Z, Liu L, Chen Z, Rothstein JD, Ransohoff RM, Gygi SP, Antel JP, Weiner HL. 2014. Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. Nat Neurosci 17:131–143.

Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, Huang D, Kidd G, Dombrowski S, Dutta R, Lee JC, Cook DN, Jung S, Lira SA, Littman DR, Ransohoff RM. 2006. Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci 9:917–924.

Chapman A, Fernandez del Ama L, Ferguson J, Kamarashev J, Wellbrock C, Hurlstone A. 2014. Heterogeneous tumor subpopulations cooperate to drive invasion. Cell Rep 8:688–695.

Chapouton P, Jagasia R, Bally-Cuif L. 2007. Adult neurogenesis in non-mammalian vertebrates. Bioessays 29:745–757.

Chen TW, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA, Orger MB, Jayaraman V, Looger LL, Svoboda K, Kim DS. 2013. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499: 295–300.

Chiu IM, Morimoto ET, Goodarzi H, Liao JT, O'Keeffe S, Phatnani HP, Muratet M, Carroll MC, Levy S, Tavazoie S, Myers RM, Maniatis T. 2013. A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. Cell Rep 4: 385–401.

Colucci-Guyon E, Tinevez JY, Renshaw SA, Herbomel P. 2011. Strategies of professional phagocytes in vivo: Unlike macrophages, neutrophils engulf only surface-associated microbes. J Cell Sci 124:3053–3059.

Curado S, Anderson RM, Jungblut B, Mumm J, Schroeter E, Stainier DY. 2007. Conditional targeted cell ablation in zebrafish: A new tool for regeneration studies. Dev Dyn 236:1025–1035.

Cusick MF, Libbey JE, Trede NS, Eckels DD, Fujinami RS. 2012. Human T cell expansion and experimental autoimmune encephalomyelitis inhibited by Lenaldekar, a small molecule discovered in a zebrafish screen. J Neuroimmunol 244:35–44.

d'Alencon CA, Pena OA, Wittmann C, Gallardo VE, Jones RA, Loosli F, Liebel U, Grabher C, Allende ML. 2010. A high-throughput chemically induced inflammation assay in zebrafish. BMC Biol 8:151.

Davidson AJ, Zon LI. 2004. The 'definitive' (and 'primitive') guide to zebrafish hematopoiesis. Oncogene 23:7233–7246.

Davis JM, Clay H, Lewis JL, Ghori N, Herbomel P, Ramakrishnan L. 2002. Real-time visualization of mycobacterium-macrophage interactions leading to initiation of granuloma formation in zebrafish embryos. Immunity 17:693–702.

Davis JM, Ramakrishnan L. 2009. The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell 136:37–49.

Deng Q, Yoo SK, Cavnar PJ, Green JM, Huttenlocher A. 2011. Dual roles for Rac2 in neutrophil motility and active retention in zebrafish hematopoietic tissue. Dev Cell 21:735–745.

Dissing-Olesen L, LeDue JM, Rungta RL, Hefendehl JK, Choi HB, MacVicar BA. 2014. Activation of neuronal NMDA receptors triggers transient ATP-mediated microglial process outgrowth. J Neurosci 34:10511–10527.

Doyon Y, McCammon JM, Miller JC, Faraji F, Ngo C, Katibah GE, Amora R, Hocking TD, Zhang L, Rebar EJ, Gregory PD, Urnov FD, Amacher SL. 2008.

Heritable targeted gene disruption in zebrafish using designed zinc-finger nucleases. Nat Biotechnol 26:702–708.

Elks PM, van Eeden FJ, Dixon G, Wang X, Reyes-Aldasoro CC, Ingham PW, Whyte MK, Walmsley SR, Renshaw SA. 2011. Activation of hypoxia-inducible factor-1alpha (Hif-1alpha) delays inflammation resolution by reducing neutrophil apoptosis and reverse migration in a zebrafish inflammation model. Blood 118:712–722.

Ellett F, Lieschke GJ. 2010. Zebrafish as a model for vertebrate hematopoiesis. Curr Opin Pharmacol 10:563–570.

Ellett F, Pase L, Hayman JW, Andrianopoulos A, Lieschke GJ. 2011. mpeg1 promoter transgenes direct macrophage-lineage expression in zebrafish. Blood 117:e49–e56.

Eyo UB, Peng J, Swiatkowski P, Mukherjee A, Bispo A, Wu LJ. 2014. Neuronal hyperactivity recruits microglial processes via neuronal NMDA receptors and microglial P2Y12 receptors after status epilepticus. J Neurosci 34:10528–10540.

Feng Y, Renshaw S, Martin P. 2012. Live imaging of tumor initiation in zebrafish larvae reveals a trophic role for leukocyte-derived PGE(2). Curr Biol 22: 1253–1259.

Feng Y, Santoriello C, Mione M, Hurlstone A, Martin P. 2010. Live imaging of innate immune cell sensing of transformed cells in zebrafish larvae: Parallels between tumor initiation and wound inflammation. PLoS Biol 8:e1000562.

Fleming A, Diekmann H, Goldsmith P. 2013. Functional characterisation of the maturation of the blood-brain barrier in larval zebrafish. PLoS One 8: e77548.

Garcia-Lecea M, Kondrychyn I, Fong SH, Ye ZR, Korzh V. 2008. In vivo analysis of choroid plexus morphogenesis in zebrafish. PLoS One 3:e3090.

Giepmans BN, Adams SR, Ellisman MH, Tsien RY. 2006. The fluorescent toolbox for assessing protein location and function. Science 312:217–224.

Gimsa U, Mitchison NA, Brunner-Weinzierl MC. 2013. Immune privilege as an intrinsic CNS property: Astrocytes protect the CNS against T-cell-mediated neuroinflammation. Mediators Inflamm 2013:320519.

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M. 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841–845.

Ginhoux F, Lim S, Hoeffel G, Low D, Huber T. 2013. Origin and differentiation of microglia. Front Cell Neurosci 7:45.

Goessling W, Allen RS, Guan X, Jin P, Uchida N, Dovey M, Harris JM, Metzger ME, Bonifacino AC, Stroncek D, Stegner J, Armant M, Schlaeger T, Tisdale JF, Zon LI, Donahue RE, North TE. 2011. Prostaglandin E2 enhances human cord blood stem cell xenotransplants and shows long-term safety in preclinical nonhuman primate transplant models. Cell Stem Cell 8:445–458.

Goldshmit Y, Sztal TE, Jusuf PR, Hall TE, Nguyen-Chi M, Currie PD. 2012. Fgf-dependent glial cell bridges facilitate spinal cord regeneration in zebrafish. J Neurosci 32:7477–7492.

Gomez-Nicola D, Fransen NL, Suzzi S, Perry VH. 2013. Regulation of microglial proliferation during chronic neurodegeneration. J Neurosci 33:2481–2493.

Grandel H, Brand M. 2013. Comparative aspects of adult neural stem cell activity in vertebrates. Dev Genes Evol 223:131–147.

Hall C, Flores MV, Chien A, Davidson A, Crosier K, Crosier P. 2009. Transgenic zebrafish reporter lines reveal conserved Toll-like receptor signaling potential in embryonic myeloid leukocytes and adult immune cell lineages. J Leukoc Biol 85:751–765.

Hall CJ, Flores MV, Oehlers SH, Sanderson LE, Lam EY, Crosier KE, Crosier PS. 2012. Infection-responsive expansion of the hematopoietic stem and progenitor cell compartment in zebrafish is dependent upon inducible nitric oxide. Cell Stem Cell 10:198–209.

Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, Griep A, Axt D, Remus A, Tzeng TC, Gelpi E, Halle A, Korte M, Latz E, Golenbock DT. 2013. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature 493:674–678.

Herbomel P, Thisse B, Thisse C. 1999. Ontogeny and behaviour of early macrophages in the zebrafish embryo. Development 126:3735–3745.

Herbomel P, Thisse B, Thisse C. 2001. Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-CSF receptor-dependent invasive process. Dev Biol 238:274–288.

Hickman SE, Kingery ND, Ohsumi TK, Borowsky ML, Wang LC, Means TK, El Khoury J. 2013. The microglial sensome revealed by direct RNA sequencing. Nat Neurosci 16:1896–1905.

Hochbaum DR, Zhao Y, Farhi SL, Klapoetke N, Werley CA, Kapoor V, Zou P, Kralj JM, Maclaurin D, Smedemark-Margulies N, Saulnier JL, Boulting GL, Straub C, Cho YK, Melkonian M, Wong GK, Harrison DJ, Murthy VN, Sabatini BL, Boyden ES, Campbell RE, Cohen AE. 2014. All-optical electrophysiology in mammalian neurons using engineered microbial rhodopsins. Nat Methods 11:825–833.

Hocking JC, Distel M, Koster RW. 2013. Studying cellular and subcellular dynamics in the developing zebrafish nervous system. Exp Neurol 242: 1–10.

Hosseini R, Lamers GE, Hodzic Z, Meijer AH, Schaaf MJ, Spaink HP. 2014. Correlative light and electron microscopy imaging of autophagy in a zebrafish infection model. Autophagy 10:1844–1857.

Huang P, Xiao A, Tong X, Zu Y, Wang Z, Zhang B. 2014. TALEN construction via "Unit Assembly" method and targeted genome modifications in zebrafish. Methods 69:67–75.

Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, Peterson RT, Yeh JR, Joung JK. 2013. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol 31:227–229.

Isogai S, Hitomi J, Yaniv K, Weinstein BM. 2009. Zebrafish as a new animal model to study lymphangiogenesis. Anat Sci Int 84:102–111.

Jones RA, Feng Y, Worth AJ, Thrasher AJ, Burns SO, Martin P. 2013. Modelling of human Wiskott-Aldrich syndrome protein mutants in zebrafish larvae using in vivo live imaging. J Cell Sci 126:4077–4084.

Kalueff AV, Echevarria DJ, Stewart AM. 2014a. Gaining translational momentum: More zebrafish models for neuroscience research. Prog Neuropsychopharmacol Biol Psychiatry 55:1–6.

Kalueff AV, Stewart AM, Gerlai R. 2014b. Zebrafish as an emerging model for studying complex brain disorders. Trends Pharmacol Sci 35:63–75.

Kaminski M, Bechmann I, Pohland M, Kiwit J, Nitsch R, Glumm J. 2012. Migration of monocytes after intracerebral injection at entorhinal cortex lesion site. J Leukoc Biol 92:31–39.

Kempermann G. 2012. New neurons for 'survival of the fittest'. Nat Rev Neurosci 13:727–736.

Kierdorf K, Erny D, Goldmann T, Sander V, Schulz C, Perdiguero EG, Wieghofer P, Heinrich A, Riemke P, Holscher C, Müller DN, Luckow B, Brocker T, Debowski K, Fritz G, Opdenakker G, Diefenbach A, Biber K, Heikenwalder M, Geissmann F, Rosenbauer F, Prinz M. 2013. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. Nat Neurosci 16:273–280.

Kobayashi N, Karisola P, Pena-Cruz V, Dorfman DM, Jinushi M, Umetsu SE, Butte MJ, Nagumo H, Chernova I, Zhu B, Sharpe AH, Ito S, Dranoff G, Kaplan GG, Casasnovas JM, Umetsu DT, Dekruyff RH, Freeman GJ. 2007. TIM-1 and TIM-4 glycoproteins bind phosphatidylserine and mediate uptake of apoptotic cells. Immunity 27:927–940.

Kokel D, Bryan J, Laggner C, White R, Cheung CY, Mateus R, Healey D, Kim S, Werdich AA, Haggarty SJ, Macrae CA, Shoichet B, Peterson RT. 2010. Rapid behavior-based identification of neuroactive small molecules in the zebrafish. Nat Chem Biol 6:231–237.

Kokel D, Rennekamp AJ, Shah AH, Liebel U, Peterson RT. 2012. Behavioral barcoding in the cloud: Embracing data-intensive digital phenotyping in neuropharmacology. Trends Biotechnol 30:421–425.

Kroehne V, Freudenreich D, Hans S, Kaslin J, Brand M. 2011. Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. Development 138:4831–4841.

Kyritsis N, Kizil C, Brand M. 2014. Neuroinflammation and central nervous system regeneration in vertebrates. Trends Cell Biol 24:128–135.

Kyritsis N, Kizil C, Zocher S, Kroehne V, Kaslin J, Freudenreich D, Iltzsche A, Brand M. 2012. Acute inflammation initiates the regenerative response in the adult zebrafish brain. Science 338:1353–1356.

Lalancette-Hebert M, Gowing G, Simard A, Weng YC, Kriz J. 2007. Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. J Neurosci 27:2596–2605.

Langenau DM, Feng H, Berghmans S, Kanki JP, Kutok JL, Look AT. 2005. Cre/lox-regulated transgenic zebrafish model with conditional myc-induced T cell acute lymphoblastic leukemia. Proc Natl Acad Sci USA 102:6068–6073.

Langenau DM, Zon LI. 2005. The zebrafish: A new model of T-cell and thymic development. Nat Rev Immunol 5:307–317.

Lewis KL, Del Cid N, Traver D. 2014. Perspectives on antigen presenting cells in zebrafish. Dev Comp Immunol 46:63–73.

Li Y, Du XF, Liu CS, Wen ZL, Du JL. 2012. Reciprocal regulation between resting microglial dynamics and neuronal activity in vivo. Dev Cell 23:1189–1202.

Lieschke GJ, Currie PD. 2007. Animal models of human disease: Zebrafish swim into view. Nat Rev Genet 8:353–367.

Livet J, Weissman TA, Kang H, Draft RW, Lu J, Bennis RA, Sanes JR, Lichtman JW. 2007. Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. Nature 450:56–62.

Long Q, Meng A, Wang H, Jessen JR, Farrell MJ, Lin S. 1997. GATA-1 expression pattern can be recapitulated in living transgenic zebrafish using GFP reporter gene. Development 124:4105–4111.

Lu Z, Elliott MR, Chen Y, Walsh JT, Klibanov AL, Ravichandran KS, Kipnis J. 2011. Phagocytic activity of neuronal progenitors regulates adult neurogenesis. Nat Cell Biol 13:1076–1083.

Lucin KM, Wyss-Coray T. 2009. Immune activation in brain aging and neurodegeneration: Too much or too little? Neuron 64:110–122.

Malafoglia V, Bryant B, Raffaeli W, Giordano A, Bellipanni G. 2013. The zebrafish as a model for nociception studies. J Cell Physiol 228:1956–1966.

Marcus RC, Easter SS Jr. 1995. Expression of glial fibrillary acidic protein and its relation to tract formation in embryonic zebrafish (Danio rerio). J Comp Neurol 359:365–381.

Mathias JR, Perrin BJ, Liu TX, Kanki J, Look AT, Huttenlocher A. 2006. Resolution of inflammation by retrograde chemotaxis of neutrophils in transgenic zebrafish. J Leukoc Biol 80:1281–1288.

Mazaheri F, Breus O, Durdu S, Haas P, Wittbrodt J, Gilmour D, Peri F. 2014. Distinct roles for BAI1 and TIM-4 in the engulfment of dying neurons by microglia. Nat Commun 5:4046.

McKeown KA, Moreno R, Hall VL, Ribera AB, Downes GB. 2012. Disruption of Eaat2b, a glutamate transporter, results in abnormal motor behaviors in developing zebrafish. Dev Biol 362:162–171.

Meijer AH, Spaink HP. 2011. Host-pathogen interactions made transparent with the zebrafish model. Curr Drug Targets 12:1000–1017.

Meng A, Tang H, Ong BA, Farrell MJ, Lin S. 1997. Promoter analysis in living zebrafish embryos identifies a cis-acting motif required for neuronal expression of GATA-2. Proc Natl Acad Sci USA 94:6267–6272.

Mickoleit M, Schmid B, Weber M, Fahrbach FO, Hombach S, Reischauer S, Huisken J. 2014. High-resolution reconstruction of the beating zebrafish heart. Nat Methods 11:919–922.

Miyanishi M, Tada K, Koike M, Uchiyama Y, Kitamura T, Nagata S. 2007. Identification of Tim4 as a phosphatidylserine receptor. Nature 450:435–439.

Mosimann C, Kaufman CK, Li P, Pugach EK, Tamplin OJ, Zon LI. 2011. Ubiquitous transgene expression and Cre-based recombination driven by the ubiquitin promoter in zebrafish. Development 138:169–177.

Moss JB, Price AL, Raz E, Driever W, Rosenthal N. 1996. Green fluorescent protein marks skeletal muscle in murine cell lines and zebrafish. Gene 173:89–98.

Munzel EJ, Becker CG, Becker T, Williams A. 2014. Zebrafish regenerate full thickness optic nerve myelin after demyelination, but this fails with increasing age. Acta Neuropathol Commun 2:77.

Muto A, Kawakami K. 2013. Prey capture in zebrafish larvae serves as a model to study cognitive functions. Front Neural Circuits 7:110.

Muto A, Ohkura M, Kotani T, Higashijima S, Nakai J, Kawakami K. 2011. Genetic visualization with an improved GCaMP calcium indicator reveals spatiotemporal activation of the spinal motor neurons in zebrafish. Proc Natl Acad Sci USA 108:5425–5430.

Nagata S, Hanayama R, Kawane K. 2010. Autoimmunity and the clearance of dead cells. Cell 140:619–630.

Nomiyama H, Hieshima K, Osada N, Kato-Unoki Y, Otsuka-Ono K, Takegawa S, Izawa T, Yoshizawa A, Kikuchi Y, Tanase S, Miura R, Kusuda J, Nakao M, Yoshie O. 2008. Extensive expansion and diversification of the chemokine gene family in zebrafish: Identification of a novel chemokine subfamily CX. BMC Genomics 9:222.

North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, Lord AM, Weber GJ, Bowman TV, Jang IH, Grosser T, Fitzgerald GA, Daley GQ, Orkin SH, Zon LI. 2007. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. Nature 447:1007–1011.

Norton WH. 2013. Toward developmental models of psychiatric disorders in zebrafish. Front Neural Circuits 7:79.

Orre M, Kamphuis W, Osborn LM, Melief J, Kooijman L, Huitinga I, Klooster J, Bossers K, Hol EM. 2014. Acute isolation and transcriptome characterization of cortical astrocytes and microglia from young and aged mice. Neurobiol Aging 35:1–14.

Page DM, Wittamer V, Bertrand JY, Lewis KL, Pratt DN, Delgado N, Schale SE, McGue C, Jacobsen BH, Doty A, Pao Y, Yang H, Chi NC, Magor BG, Traver D. 2013. An evolutionarily conserved program of B-cell development and activation in zebrafish. Blood 122:e1–e11.

Panula P, Chen YC, Priyadarshini M, Kudo H, Semenova S, Sundvik M, Sallinen V. 2010. The comparative neuroanatomy and neurochemistry of zebrafish CNS systems of relevance to human neuropsychiatric diseases. Neurobiol Dis 40:46–57.

Paquet D, Bhat R, Sydow A, Mandelkow EM, Berg S, Hellberg S, Falting J, Distel M, Koster RW, Schmid B, Haass C. 2009. A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation. J Clin Invest 119:1382–1395.

Pardo-Martin C, Chang TY, Koo BK, Gilleland CL, Wasserman SC, Yanik MF. 2010. High-throughput in vivo vertebrate screening. Nat Methods 7:634–636.

Park D, Tosello-Trampont AC, Elliott MR, Lu M, Haney LB, Ma Z, Klibanov AL, Mandell JW, Ravichandran KS. 2007. BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. Nature 450: 430–434.

Park HC, Mehta A, Richardson JS, Appel B. 2002. olig2 is required for zebrafish primary motor neuron and oligodendrocyte development. Dev Biol 248:356–368.

Peravali R, Gehrig J, Giselbrecht S, Lutjohann DS, Hadzhiev Y, Muller F, Liebel U. 2011. Automated feature detection and imaging for high-resolution screening of zebrafish embryos. Biotechniques 50:319–324.

Peri F, Nusslein-Volhard C. 2008. Live imaging of neuronal degradation by microglia reveals a role for v0-ATPase a1 in phagosomal fusion in vivo. Cell 133:916–927.

Peterson RT, Link BA, Dowling JE, Schreiber SL. 2000. Small molecule developmental screens reveal the logic and timing of vertebrate development. Proc Natl Acad Sci USA 97:12965–12969.

Petrie TA, Strand NS, Tsung-Yang C, Rabinowitz JS, Moon RT. 2014. Macrophages modulate adult zebrafish tail fin regeneration. Development 141: 2581–2591.

Pisharath H, Rhee JM, Swanson MA, Leach SD, Parsons MJ. 2007. Targeted ablation of beta cells in the embryonic zebrafish pancreas using E. coli nitroreductase. Mech Dev 124:218–229.

Pope HM, Voigt MM. 2014. Peripheral glia have a pivotal role in the initial response to axon degeneration of peripheral sensory neurons in zebrafish. PLoS One 9:e103283.

Postlethwait JH, Woods IG, Ngo-Hazelett P, Yan YL, Kelly PD, Chu F, Huang H, Hill-Force A, Talbot WS. 2000. Zebrafish comparative genomics and the origins of vertebrate chromosomes. Genome Res 10:1890–1902.

Powell C, Grant AR, Cornblath E, Goldman D. 2013. Analysis of DNA methylation reveals a partial reprogramming of the Muller glia genome during retina regeneration. Proc Natl Acad Sci USA 110:19814–19819.

Prinz M, Priller J. 2014. Microglia and brain macrophages in the molecular age: From origin to neuropsychiatric disease. Nat Rev Neurosci 15:300–312.

Quintana FJ, Iglesias AH, Farez MF, Caccamo M, Burns EJ, Kassam N, Oukka M, Weiner HL. 2010. Adaptive autoimmunity and Foxp3-based immunoregulation in zebrafish. PLoS One 5:e9478.

Ransohoff RM, Perry VH. 2009. Microglial physiology: Unique stimuli, specialized responses. Annu Rev Immunol 27:119–145.

Ravichandran KS, Lorenz U. 2007. Engulfment of apoptotic cells: Signals for a good meal. Nat Rev Immunol 7:964–974.

Reddien PW, Horvitz HR. 2004. The engulfment process of programmed cell death in caenorhabditis elegans. Annu Rev Cell Dev Biol 20:193–221.

Renshaw SA, Trede NS. 2012. A model 450 million years in the making: Zebrafish and vertebrate immunity. Dis Model Mech 5:38–47.

Reutelingsperger CP, van Heerde WL. 1997. Annexin V, the regulator of phosphatidylserine-catalyzed inflammation and coagulation during apoptosis. Cell Mol Life Sci 53:527–532.

Ridges S, Heaton WL, Joshi D, Choi H, Eiring A, Batchelor L, Choudhry P, Manos EJ, Sofla H, Sanati A, Welborn S, Agarwal A, Spangrude GJ, Miles RR, Cox JE, Frazer JK, Deininger M, Balan K, Sigman M, Müschen M, Perova T, Johnson R, Montpellier B, Guidos CJ, Jones DA, Trede NS. 2012. Zebrafish screen identifies novel compound with selective toxicity against leukemia. Blood 119:5621–5631.

Rihel J, Prober DA, Arvanites A, Lam K, Zimmerman S, Jang S, Haggarty SJ, Kokel D, Rubin LL, Peterson RT, Schier AF. 2010. Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. Science 327:348–351.

Robertson AL, Holmes GR, Bojarczuk AN, Burgon J, Loynes CA, Chimen M, Sawtell AK, Hamza B, Willson J, Walmsley SR, Anderson SR, Coles MC, Farrow SN, Solari R, Jones S, Prince LR, Irimia D, Rainger GE, Kadirkamanathan V, Whyte MK, Renshaw SA. 2014. A zebrafish compound screen reveals modulation of neutrophil reverse migration as an anti-inflammatory mechanism. Sci Transl Med 6:225ra29.

Sander JD, Cade L, Khayter C, Reyon D, Peterson RT, Joung JK, Yeh JR. 2011. Targeted gene disruption in somatic zebrafish cells using engineered TALENs. Nat Biotechnol 29:697–698.

Sauer B. 1998. Inducible gene targeting in mice using the Cre/lox system. Methods 14:381–392.

Saxena M, Yeretssian G. 2014. NOD-like receptors: Master regulators of inflammation and cancer. Front Immunol 5:327.

Schiffer NW, Broadley SA, Hirschberger T, Tavan P, Kretzschmar HA, Giese A, Haass C, Hartl FU, Schmid B. 2007. Identification of anti-prion compounds as efficient inhibitors of polyglutamine protein aggregation in a zebrafish model. J Biol Chem 282:9195–9203.

Schlueter PJ, Peterson RT. 2009. Systematizing serendipity for cardiovascular drug discovery. Circulation 120:255–263.

Schmid B, Haass C. 2013. Genomic editing opens new avenues for zebrafish as a model for neurodegeneration. J Neurochem 127:461–470.

Schmid B, Hruscha A, Hogl S, Banzhaf-Strathmann J, Strecker K, van der Zee J, Teucke M, Eimer S, Hegermann J, Kittelmann M, Kremmer E, Cruts M, Solchenberger B, Hasenkamp L, van Bebber F, Van Broeckhoven C, Edbauer D, Lichtenthaler SF, Haass C. 2013. Loss of ALS-associated TDP-43 in zebrafish causes muscle degeneration, vascular dysfunction, and reduced motor neuron axon outgrowth. Proc Natl Acad Sci USA 110:4986–4991.

Schwartz M, Kipnis J, Rivest S, Prat A. 2013. How do immune cells support and shape the brain in health, disease, and aging? J Neurosci 33:17587–17596.

Serhan CN, Savill J. 2005. Resolution of inflammation: The beginning programs the end. Nat Immunol 6:1191–1197.

Shaner NC, Steinbach PA, Tsien RY. 2005. A guide to choosing fluorescent proteins. Nat Methods 2:905–909.

Shiau CE, Monk KR, Joo W, Talbot WS. 2013. An anti-inflammatory NOD-like receptor is required for microglia development. Cell Rep 5:1342–1352.

Sieger D, Moritz C, Ziegenhals T, Prykhozhij S, Peri F. 2012. Long-range $Ca2+$ waves transmit brain-damage signals to microglia. Dev Cell 22:1138–1148.

Sjollema KA, Schnell U, Kuipers J, Kalicharan R, Giepmans BN. 2012. Correlated light microscopy and electron microscopy. Methods Cell Biol 111:157–173.

Skaggs K, Goldman D, Parent JM. 2014. Excitotoxic brain injury in adult zebrafish stimulates neurogenesis and long-distance neuronal integration. Glia 62:2061–2079.

Stockhammer OW, Zakrzewska A, Hegedus Z, Spaink HP, Meijer AH. 2009. Transcriptome profiling and functional analyses of the zebrafish embryonic innate immune response to Salmonella infection. J Immunol 182:5641–5653.

Stoletov K, Kato H, Zardouzian E, Kelber J, Yang J, Shattil S, Klemke R. 2010. Visualizing extravasation dynamics of metastatic tumor cells. J Cell Sci 123:2332–2341.

Streisinger G, Walker C, Dower N, Knauber D, Singer F. 1981. Production of clones of homozygous diploid zebra fish (Brachydanio rerio). Nature 291:293–296.

Streit WJ. 2006. Microglial senescence: Does the brain's immune system have an expiration date? Trends Neurosci 29:506–510.

Suster ML, Sumiyama K, Kawakami K. 2009. Transposon-mediated BAC transgenesis in zebrafish and mice. BMC Genomics 10:477.

Teh C, Chudakov DM, Poon KL, Mamedov IZ, Sek JY, Shidlovsky K, Lukyanov S, Korzh V. 2010. Optogenetic in vivo cell manipulation in KillerRed-expressing zebrafish transgenics. BMC Dev Biol 10:110.

Tobin DM, May RC, Wheeler RT. 2012. Zebrafish: A see-through host and a fluorescent toolbox to probe host-pathogen interaction. PLoS Pathog 8:e1002349.

Torraca V, Masud S, Spaink HP, Meijer AH. 2014. Macrophage-pathogen interactions in infectious diseases: New therapeutic insights from the zebrafish host model. Dis Model Mech 7:785–797.

Trede NS, Langenau DM, Traver D, Look AT, Zon LI. 2004. The use of zebrafish to understand immunity. Immunity 20:367–379.

Vajn K, Suler D, Plunkett JA, Oudega M. 2014. Temporal profile of endogenous anatomical repair and functional recovery following spinal cord injury in adult zebrafish. PLoS One 9:e105857.

van der Vaart M, van Soest JJ, Spaink HP, Meijer AH. 2013. Functional analysis of a zebrafish myd88 mutant identifies key transcriptional components of the innate immune system. Dis Model Mech 6:841–854.

van Ham TJ, Brady CA, Kalicharan RD, Oosterhof N, Kuipers J, Veenstra-Algra A, Sjollema KA, Peterson RT, Kampinga HH, Giepmans BN. 2014. Intravital correlated microscopy reveals differential macrophage and microglial dynamics during resolution of neuroinflammation. Dis Model Mech 7:857–869.

van Ham TJ, Kokel D, Peterson RT. 2012. Apoptotic cells are cleared by directional migration and elmo1-dependent macrophage engulfment. Curr Biol 22:830–836.

van Ham TJ, Mapes J, Kokel D, Peterson RT. 2010. Live imaging of apoptotic cells in zebrafish. FASEB J 24:4336–4342.

Volkman HE, Pozos TC, Zheng J, Davis JM, Rawls JF, Ramakrishnan L. 2010. Tuberculous granuloma induction via interaction of a bacterial secreted protein with host epithelium. Science 327:466–469.

Walters KB, Green JM, Surfus JC, Yoo SK, Huttenlocher A. 2010. Live imaging of neutrophil motility in a zebrafish model of WHIM syndrome. Blood 116:2803–2811.

Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, Cella M, Barrow AD, Diamond MS, Colonna M. 2012. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. Nat Immunol 13:753–760.

Weber T, Koster R. 2013. Genetic tools for multicolor imaging in zebrafish larvae. Methods 62:279–291.

Wieghofer P, Knobeloch KP, Prinz M. 2015. Genetic targeting of microglia. Glia 63:1–22.

Wienholds E, van Eeden F, Kosters M, Mudde J, Plasterk RH, Cuppen E. 2003. Efficient target-selected mutagenesis in zebrafish. Genome Res 13: 2700–2707.

Willett CE, Cortes A, Zuasti A, Zapata AG. 1999. Early hematopoiesis and developing lymphoid organs in the zebrafish. Dev Dyn 214:323–336.

Willett CE, Kawasaki H, Amemiya CT, Lin S, Steiner LA. 2001. Ikaros expression as a marker for lymphoid progenitors during zebrafish development. Dev Dyn 222:694–698.

Wilson SW, Brand M, Eisen JS. 2002. Patterning the zebrafish central nervous system. Results Probl Cell Differ 40:181–215.

Wittamer V, Bertrand JY, Gutschow PW, Traver D. 2011. Characterization of the mononuclear phagocyte system in zebrafish. Blood 117:7126–7135.

Wittmann C, Reischl M, Shah AH, Mikut R, Liebel U, Grabher C. 2012. Facilitating drug discovery: An automated high-content inflammation assay in zebrafish. J Vis Exp e4203.

Woodfin A, Voisin MB, Beyrau M, Colom B, Caille D, Diapouli FM, Nash GB, Chavakis T, Albelda SM, Rainger GE, Meda P, Imhof BA, Nourshargh S. 2011. The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo. Nat Immunol 12:761–769.

Yaniv K, Isogai S, Castranova D, Dye L, Hitomi J, Weinstein BM. 2006. Live imaging of lymphatic development in the zebrafish. Nat Med 12:711–716.

Yoo SK, Starnes TW, Deng Q, Huttenlocher A. 2011. Lyn is a redox sensor that mediates leukocyte wound attraction in vivo. Nature 480:109–112.

Yu PB, Deng DY, Lai CS, Hong CC, Cuny GD, Bouxsein ML, Hong DW, McManus PM, Katagiri T, Sachidanandan C, Kamiya N, Fukuda T, Mishina Y, Peterson RT, Bloch KD. 2008a. BMP type I receptor inhibition reduces heterotopic [corrected] ossification. Nat Med 14:1363–1369.

Yu PB, Hong CC, Sachidanandan C, Babitt JL, Deng DY, Hoyng SA, Lin HY, Bloch KD, Peterson RT. 2008b. Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. Nat Chem Biol 4:33–41.

Zhang R, Han P, Yang H, Ouyang K, Lee D, Lin YF, Ocorr K, Kang G, Chen J, Stainier DY, Yelon D, Chi NC. 2013. In vivo cardiac reprogramming contributes to zebrafish heart regeneration. Nature 498:497–501.

Zhang S, Cui P. 2014. Complement system in zebrafish. Dev Comp Immunol 46:3–10.

Zon LI, Peterson RT. 2005. In vivo drug discovery in the zebrafish. Nat Rev Drug Discov 4:35–44.