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REVIEW

Role of hemocytes in invertebrate adult neurogenesis and brain repair

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Abstract

The repair of lesions of the central nervous system (CNS) varies widely throughout the animal kingdom. At the level of neuronal replacement lie the major differences in CNS regeneration. At one extreme are the amniote vertebrates (reptile, avian and mammalian groups), which have very limited capacity for neuronal replacement, and therefore for neural regeneration; at the other extreme, animals such as planarians (flatworms) and colonial tunicates can repair their entire CNS after major injuries. These differences can be attributed to the abundance of multipotent and/or pluripotent stem cells and/or undifferentiated precursors among the general cell population. In this review we discuss recent advancements in knowledge of regeneration of the CNS of invertebrates. We focus on ascidians, which are a sister group of vertebrates, but we also address other invertebrate groups. Because neurogenesis is central to the events that allow regeneration of the adult CNS, we address this issue focusing on crustaceans, which have provided a paradigm to study the mechanisms underlying this phenomenon. The attraction of hemocytes toward a neurogenic niche and respecification of these cells toward a neural fate has been strongly suggested. Based on recent and emerging research, we suggest that cells of the blood lineage are not only associated with the roles that are generally attributed to them, but are the cells that either signal other cell types to differentiate into neural cells, or even eventually themselves transdifferentiate into neural cells.

Key Words: neuroregeneration; neurogenesis; blood cells; stem cells; hematopoietic tissue; ascidians; crustaceans

Introduction

The ability of many animals to regenerate their whole body or substantial parts of the body is a remarkable biological phenomenon that is non-uniformly represented in different phyla. Mammals, for example, have a limited capacity to regenerate and restore tissues and organs. Mechanisms associated with natural regeneration include dedifferentiation, reprogramming and transdifferentiation (Jopling *et al*., 2011). Transdifferentiation, the switch of lineages to create another cell type, is often illustrated by the change of pancreas exocrine cells to endocrine Beta cells following a lesion (Bouwens, 1998). However, as will be emphasized in the following section and elsewhere, it is now believed that certain normal phenomena, such as adult neurogenesis, may involve transdifferentiation (see Mezey and Brownstein, 2015, for a review).

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The role of mesenchymal stem cells and blood cells in the repair of lesions in the central nervous system of vertebrates

Mesenchymal stem cells (MSCs) were originally identified as a population of fibroblastic cells that are found in the bone marrow of vertebrates, and that are distinct from the hematopoietic lineage (Friedenstein *et al*., 1976). However, MSCs, considered to be multipotent, can also reside in other adult tissues, including circulating blood cells (Kuznetsov *et al*., 2001; Anker *et al*., 2003; Miura *et al*., 2003; Rosada *et al*., 2003; Salingcarnboriboon *et al*., 2003; Vandenabeele *et al*., 2003; Igura *et al*., 2004; Seo *et al*., 2004; Tsai *et al*., 2004; Toma *et al*., 2009; Delorme *et al*., 2010). A series of studies have proposed that MSCs have a higher potential for differentiation than previously thought, including the ability to form both endodermal and ectodermal tissue (Petersen *et al*., 1999; Mezey *et al*., 2000; Sanchez-Ramos *et al*., 2000; Woodbury *et al*., 2000; Krause *et al*., 2001; Woodbury *et al*., 2002; for a review see Mezey, 2007).

In recent years, some investigators have challenged the notion that multipotent stem cells are restricted in their potency to the formation of cell types that have originated from only one embryonic germ layer. Several authors have reported that different stem cells can form cell types of other germ layers, a process termed transdifferentiation. Dedifferentiation can also explain the regeneration of body structures. During tail regeneration in salamanders, for example, mature muscle fibers lose their myofibrillar structure, their nuclei become enlarged, and mononucleate cells proliferate to populate the specific mass of cells that are capable of growth and regeneration, the blastema. Endogenous muscle fibers lying next to the site of experimental amputation dedifferentiate and form mononucleate cells, which constitute a proportion of the blastema (Echeverri and Tanaka, 2002).

In addition to being regarded as possible candidates for the treatment of diseases affecting mesodermal tissues, due to the functional recovery observed in various animal models with neural lesions, MSCs are being considered as potential candidates for neurological treatments. Three main hypotheses have been proposed to explain MSC-mediated neurogenesis and neural repair: 1) transdifferentiation (Sanchez-Ramos *et al*., 2000; Woodbury *et al*., 2000; Mezey *et al*., 2000); 2) cell fusion (Terada *et al*., 2002); and 3) paracrine activity through the release of soluble factors (Urdzíková *et al*., 2006). While there is evidence for all three phenomena, the debate over the degree of contribution of each of these models continues.

Stem cells derived from the umbilical cord have also been the subject of research focusing on repairing lesions in the brain of mammals. Human umbilical-cord blood cells proved to be able to differentiate into neurons *in vitro* (Sanchez-Ramos *et al*., 2001) and when transplanted into the developing rat brain (Zigova *et al*., 2002). In adult rats, peripheral-blood progenitor cells reduced behavioral and functional deficits associated with cerebral infarction (Willing *et al*., 2003). More recently, the umbilical cord matrix has been confirmed as a suitable source of MSCs for applications in neurodegeneration, due to their primordial nature, neural-like plasticity, and readily availability with no significant ethical concerns (Leite *et al.*, 2014; Frausin *et al.*, 2015). Therefore, future therapies based on the use of peripheral blood cells for treatment of CNS diseases are a real possibility.

Stem cells in invertebrates

Life-long growth without fixed limits is typical of some evolutionarily very successful groups of aquatic invertebrates, such as echinoderms, bivalve molluscs and decapod crustaceans. These animals continue to enlarge their organs as adults and can regenerate lost appendages and organs, which is in sharp contrast to mammals and most insects. Interestingly, according to specialized literature, echinoderms (Ebert, 2008), bivalve molluscs (Schöne *et al*., 2005) and decapods (Vogt, 2010, 2012a) only rarely develop neoplastic and age-related diseases, although some species can live for more than 100 years. Maximum ages range from 40 days to 72 years for decapods, 1 to 375 years for bivalves, and almost 200 years for echinoderms. There are indications that their stem-cell systems have co-evolved with their successful environment-adapted life histories, suggesting that study of their features may offer new insights into stem-cell biology. In fact, several types of adult stem cells, as well as some types of mature cells that are capable of dedifferentiating into multipotent progenitor cells have been identified (Vogt, 2012b).

Hydrozoans (Phylum Cnidaria) have a great capacity for regeneration, and the presence of multipotent stem cells in these organisms plays an important role in their normal development and also in regeneration (Gierer, 1977; Chandebois, 1976). Cnidarians are phylogenetically basal members of the animal kingdom and show unlimited regeneration capacity and immortality. Immortality can be described as the asexual mode of reproduction that requires cells with an unlimited self-renewal capacity (Watanabe *et al*., 2009). Cnidarian stem cells can give rise to a number of differentiated cell types, including neuronal and germ cells. Their phylogenetic position, at the base of the metazoan branch of the evolutionary tree, makes them an important link in clarifying the mechanisms of stem-cell biology that are common to both animals and plants (Watanabe *et al*., 2009). Among many stem-cell markers in cnidarians, the transcription factor forkhead box O (FoxO) has been shown to modulate the proliferation capacity of stem cells in order to regulate the lifespan and delay aging (Boehm *et al*., 2013).

Knowledge of the biology of stem cells of the blood-cell lineage has grown since the discovery that they can be isolated from various adult organs, cultured, made to differentiate into different cell types, and put back into host organisms. The special attention to stem cells has also resulted in lines of research aiming at determining the evolutionary origin and subsequent modifications of this cell type. In flatworms, undifferentiated stem cells called neoblasts are morphologically comparable to vertebrate hemocytoblasts (Andrew,

1965), and are able to differentiate into all cell types during normal postembryonic development and regeneration (Ehlers, 1985). Stem-cell research has used *Drosophila* (Hartenstein, 2013) to genetically tag individual stem cells and to observe their ability to self-renew for long periods (Morrison and Spradling, 2008). This period may range from 7 (López-Oneiva *et al*., 2008) to 25 days (Nystul and Spradling, 2007). The resident stem cells from different sources, including stromal (López-Oneiva *et al*., 2008) and epithelial niche (Nystul and Spradling, 2007) are maintained in an undifferentiated state using a short-range intercellular signal. These mechanisms of stem-cell maintenance are important to understand both the regulation of homeostasis and the possible alterations that may occur during adulthood (Morrison and Spradling, 2008).

The classification of blood cells of invertebrates, the hemocytes, is not yet uniform, and many different terminologies are used to identify these cells. In some cases, differences in terminology exist even for the same species. Hartenstein (2006) attempted to reconcile the terminology for invertebrate blood cells with that used for vertebrates, based on highly conserved molecular pathways involved in the development and function of these cells. Among the characteristics of both vertebrate and invertebrate blood cells, blood-cell lineages diverge from a common type of progenitor cell, the blood stem cell (Hartenstein, 2006).

In general, the classification of circulating hemocytes in invertebrates is based mainly on the amount of granules in the cytoplasm and the nucleus/cytoplasm ratio (Johansson *et al*., 2000). In crustaceans, including the crab *Ucides cordatus* (Chaves da Silva *et al*., 2010), three types of hemocytes can be identified: hyalinocytes (agranular cells), semigranular cells (cells with small granules), and granular cells (cells with large granules) (Bauchau, 1981; Söderhäll and Smith, 1983; Martin and Graves, 1985; Hose *et al*., 1990; Johansson *et al*., 2000; van de Braak *et al*., 2002;. Battison *et al*., 2003). The hyalinocytes are considered the immature blood cell type (Cochennec-Laureau *et al*., 2003).

In ascidians (Phylum Chordata, Class Ascidiacea), the largest and the most diverse class of the Subphylum Tunicata, which is considered a sister-group of vertebrates, the blood stem cell is termed the hemoblast and shares many characteristics, specification mechanisms and regulatory pathways with the blood stem cells of vertebrates (Sawada *et al*., 1993; Cima *et al*., 2001; Ballarin and Cima, 2005; De Barros *et al*., 2007; Ballarin and Kawamura, 2009; De Barros *et al*., 2009; De Barros *et al*., 2014). Hemoblasts have been extensively studied in the colonial ascidian *Botryllus primigenus*, and a few studies have examined solitary species.

In a colonial ascidian, two types of hemoblasts have been described, somatic and germ-line hemoblasts. They are morphologically similar and show multipotent capacity, but have different cell markers (Kawamura and Sunanaga, 2010). In the solitary ascidian *Styela plicata*, hemoblasts were shown to be spherical cells with a high nucleus/cytoplasm ratio. They appear either circulating (Figs 1A, B) or in the hematopoietic tissue (Fig. 1C). Their cytoplasm contains few or no granules and few visible organelles, and the nucleus contains one or more nucleoli (De Barros *et al*., 2009, 2014). At most, they comprise 1-2 % of the celomic population (Wright, 1981).

Hemoblasts can be found within the hemolymph or aggregated in compartments that maintain and regulate their fates in ascidians, the so-called hematopoietic niches or stem-cell nodules, such as the branchial vessels, intestine submucosa, and vascular ampullae of colonial ascidians (Ermak, 1976; Voskoboynik *et al*., 2008; Rinkevich *et al*., 2013). Although the clear morphological characteristics of ascidian blood progenitors have been difficult to identify reliably, due to a lack of maintenance and differentiation markers or of reports using molecular-biology techniques, nevertheless, important markers do exist, many of which are described in colonial ascidians.

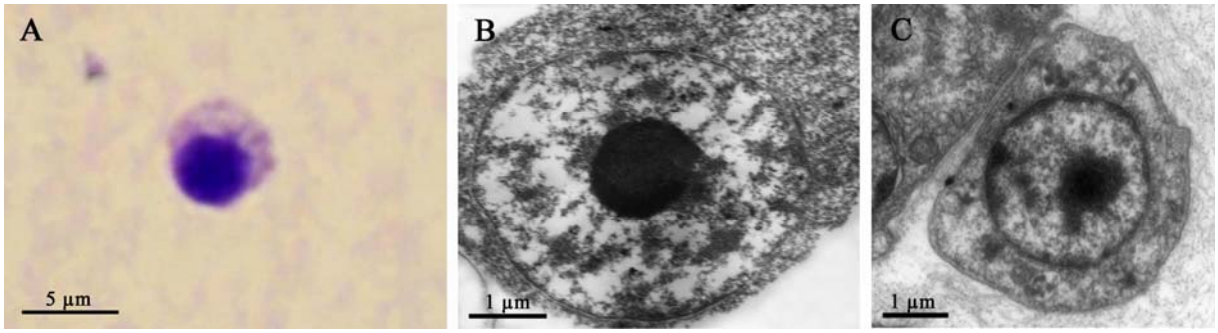

Fig. 1 Ascidian hemoblasts. (A) From *Phallusia nigra*, observed by light microscopy after removal from circulating hemolymph (stained with toluidine blue). (B) From *Styela plicata*, observed by transmission electron microscopy after removal from circulating hemolymph. (C) From *S. plicata*, obtained from hematopoietic tissue located in the intestine submucosa.

Ascidian hemoblasts express a CD34-like antigen (Medina *et al*., 2014). CD34 is a human hematopoietic molecular marker conserved in mammals, and is a glycoprotein involved in adhesion mechanisms (Gallacher *et al*., 2000). Although Ballarin and Cima (2005) reported the presence of the hematopoietic marker CD34 in *Botryllus schlosseri*, recently, Braden *et al.* (2014) did not find any homologue gene to CD34 in the same species. Therefore, it remains an open question whether this gene is evolutionarily conserved in ascidians, since genetic sequencing has not yet been conducted in many ascidian species.

Based on investigations conducted on both solitary and colonial ascidians, germ-line cells strongly express a *vasa* homologue gene involved in the formation or development of germ cells (Fujimura and Takamura, 2000; Brown and Swalla, 2007). Recently, the Piwi ascidian stem-cell marker was identified in the colonial tunicate *Botrylloides leachi* (Rinkevich *et al*., 2010). Piwi belongs to the evolutionarily conserved PIWI/Argonaute superfamily of RNA interface effector proteins, which are essential for both self-renewal and maintenance of germ-line and somatic stem cells in various multicellular organisms (Cox *et al*., 1998; Carmell *et al*., 2007; Brown *et al*., 2009).

Hemoblasts play a key role in tissue renewal during reproduction and regeneration. Some of them differentiate into somatic-lineage cells, such as endodermal multipotent epithelial, cardiac, muscle and blood cells (Burighel and Cloney, 1997); and others into germ cells, known to regenerate the whole body of botryllid ascidians (Sunanaga *et al*., 2006; Rinkevich *et al*., 2013). This phenomenon can be explained when the proportion of hematopoietic stem cells found in humans (Pike-Overzet *et al*., 2009) is compared with that in ascidians: humans have 1 stem cell per 100,000 circulating blood cells, whereas botryllid ascidians have 1 per 5,000 (Laird *et al*., 2005; Brown *et al*., 2009).

Hematopoietic cells in invertebrates

Although the concept of a niche was initially proposed by Schofield (1978) for mouse hematopoiesis, it is probably fair to say that the *in vivo* signaling properties of niche cells were first discovered in invertebrate germline stem cells, including *Drosophila* and *Caenorhabditis elegans* (Xie and Spradling, 2000; Kiger *et al*., 2001; Crittenden *et al*., 2002). Studies on the regulatory functions of local cell types in hematopoietic niches of invertebrates have been reviewed by Adams and Scadden (2006). Four main ideas derived from invertebrates were described in the review: 1) the number of stem cells in a niche is tightly regulated; 2) physical interaction among heterologous types of cells is important for the maintenance of the stem-cell state; 3) products of the niche provide the molecular basis for physical interactions and a balance of inhibitory and stimulatory signals governing stem-cell number and function; and 4) niche occupancy can impose 'stem cell-like' characteristics on some cells, even if they are not stem cells. Therefore, the authors suggested that examination of the pathways and perhaps the structural components of invertebrate stem-cell niches may improve the understanding of how the specialized microenvironment can affect mammalian stem cells (Adams and Scadden, 2006).

The hematopoietic tissue in invertebrates (HPT) is responsible for the production and release of circulating blood cells (hemocytes). In crustaceans the HPT is composed of a number of ovoid lobules, which form a thin sheet on the dorsal part of the stomach (foregut), and are surrounded by connective tissue (Chaga *et al*., 1995; Martin *et al*., 1993; Johansson *et al*., 2000). This location makes crustaceans a suitable model for studying hematopoiesis, because the tissue can be readily isolated, and the proliferation of stem cells and their differentiation can be studied both *in vivo* and *in vitro*. In many crustaceans, the various types of hematopoietic cells are mainly characterized by their morphology as seen under either light or electron microscopy, and are described based on the amount of granules and/or through biochemical analyses (Söderhäll and Smith, 1983; Johansson *et al*., 2000). However, the characterization of the hematopoietic cells depends on the species. According to van de Braak *et al*. (2002), in the shrimp *Penaeus monodon,* the type 1 cells are the presumed precursor cells that originate a large- and a small-granular hemocyte, called type 2 and type 3 cells, respectively. The type 4 cells show typical features of interstitial cells. In contrast, five different cell types have been found in crayfish: the type 1 and 2 cells are the main proliferating cells in the HPT, whereas the other cell types are precursors of granular and semigranular cells (Lin and Soderhall, 2011).

Recently, Noonin *et al*. (2012) demonstrated that the hematopoietic system in crustaceans is far more extensive than previously recognized. A specialized region of the hematopoietic system, called the anterior proliferation center (APC) and located near the brain, is proposed to constitute a multipotent stem-cell center. In fact, the APC is only one part of a much more extensive hematopoietic system. It contains actively proliferating cells in the anterior part of the tissue near the area linking the HPT to the brain (Fig. 2).

Anatomical and morphological analyses showed that the APC lies within the *cor frontale*, or auxiliary heart, which pumps hemolymph to the brain and eyes through the cerebral and ophthalmic arteries, respectively. Interestingly, in both areas there is neurogenesis. These data, together with the fact that BrdU-positive cells were observed within the dorsal median artery, which comes from the posterior HPT forward to the brain, suggest that APC cells are multipotent stem cells (Chaves da Silva *et al*., 2013). The hypothesis is that APC cells probably establish a communication with the brain in order to populate the neurogenic niche located in the brain of crustaceans (Sullivan *et al*., 2007), and possibly to interact with resident niche cells by releasing growth factors or even to transdifferentiate into neural progenitor cells (Chaves da Silva *et al*., 2013). Although there is no evidence for transdifferentiation of hemocytes into neural cells, the study of Benton *et al.* (2014) identified hemocytes as a source of adult-born neurons in crayfish, and demonstrated that the immune system is a key contributor to adult neurogenesis.

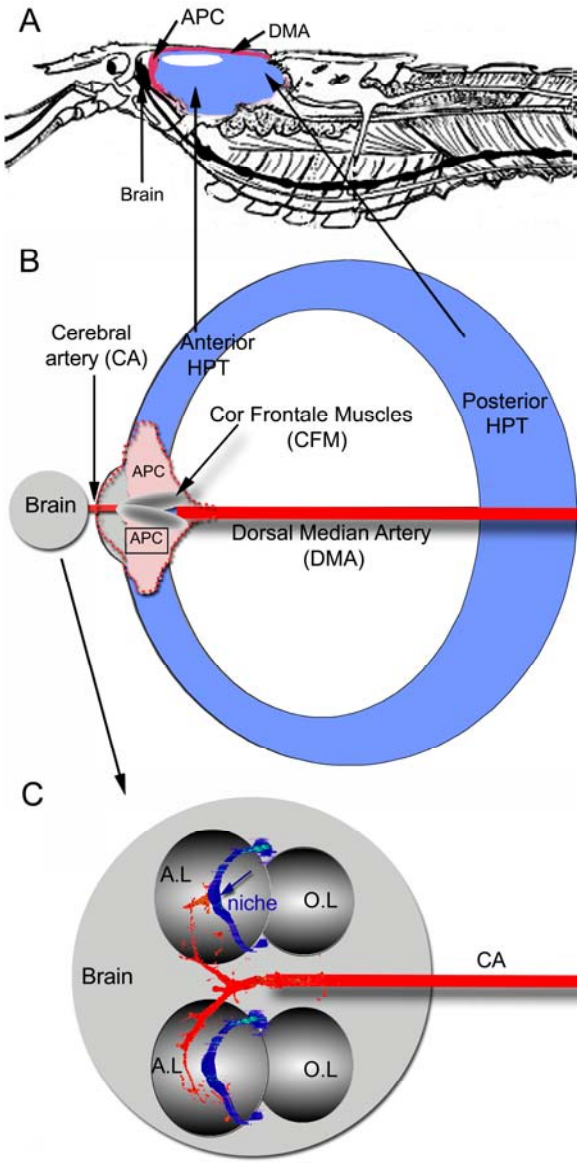

Fig. 2 Scheme of the crayfish *Procambarus clarkii*, showing the hematopoietic system. (A) The posterior hematopoietic tissue (HPT-blue) extends bilaterally toward the head, raising the anterior proliferation center (APC-red). (B) Scheme showing only the hematopoietic system and the brain. The dorsal median artery (DMA-red line) links the posterior HPT (blue) to APC (red), which is between the posterior-HPT and the brain. The APC surrounds the *cor frontale* muscles (CFM), within the *cor frontale*, an auxiliary heart that pumps hemolymph toward the brain through the cerebral artery (CA) (Image modified from Chaves da Silva *et al.,* 2013). (C) Scheme of brain (illustrated in B) showing the cerebral artery (CA) entering the brain and bilaterally accessing the neurogenic niche (niche) on the surface of the accessory lobe (AL). (OL) olfactory lobe.

Adult neurogenesis in invertebrates

Neurogenesis persists in the adult brains of various animals, and is a common phenomenon that is not correlated with their phylogeny or the overall complexity of their brains. Based on the review by Cayre *et al*. (2002), it is very likely that similar processes underlie neurogenesis in the adult brain of both invertebrates and vertebrates. In vertebrates, adult neurogenesis has been widely explored in specific regions of the brain, including the hippocampus of mammals (Altman and Das, 1965, Gheusi and Lledo, 2007; Ninkovic *et al*., 2007). Among invertebrates, adult neurogenesis has been analyzed mainly in arthropods, i.e. in several species of insects and decapod crustaceans, with no information available for numerous other taxa and countless species (Schmidt, 2007).

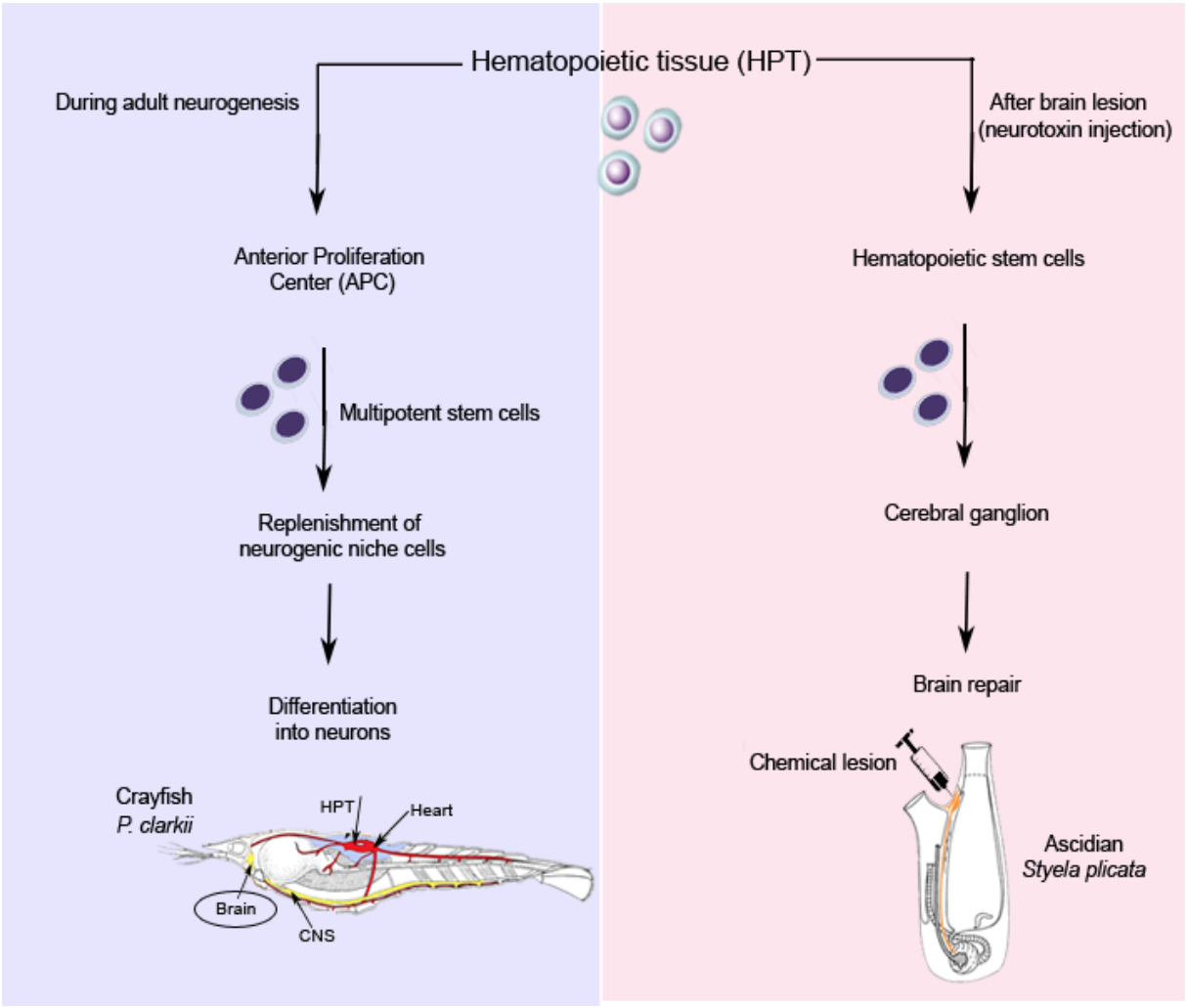

Fig. 3 Scheme of the role of hematopoietic stem cells in the crustacean *Procambarus clarkii* during adult neurogenesis and in the ascidian *Styela plicata* during brain repair. In the crayfish, the anterior proliferation center (APC), a part of the hematopoietic tissue, produces multipotent stem cells that have access to the brain and populate the neurogenic niche, possibly differentiating into neurons. In the ascidian, after the injection of a neurotoxin into the brain, the hematopoietic stem cells migrate to the cerebral ganglion for repair.

In insect brains, adult neurogenesis only occurs in the mushroom bodies, which are higher-order multimodal integration centers (Cayre *et al*., 1994, 1996; Gu *et al*., 1999; Dufour and Gadenne, 2006; Cayre *et al*., 2007). However, neurogenesis does not occur in many insect groups, such as Dictyoptera and Acrididae (Cayre *et al*., 1996) or in honeybees (Fahrbach *et al*., 1995); and lasts only several days into adulthood in cockroaches (Gu *et al*., 1999).

In decapod crustaceans, new neurons are continuously produced in two brain areas: the optic lobes and the central olfactory system (Harzsch and Dawirs, 1996; Schmidt, 1997; Sandeman *et al*., 1998; Sullivan *et al*., 2007), which makes them useful animal models for providing insights into adult neurogenesis. In the crayfish *Procambarus clarkii* (phylum Arthropoda; subphylum Crustacea), adult neurogenesis involves at least three generations of precursor cells (Sullivan *et al*., 2007). The first-generation precursor cells reside in a vascularized neurogenic niche. These bipolar niche cells also provide a stream along which their progeny migrate. The second-generation cells are migratory precursors that move toward the proliferation (medial and lateral) zones where the neuron cell bodies are grouped in clusters (clusters 9 and 10). The progeny differentiate into cluster 9 (local) and cluster 10 (projection) olfactory neurons, respectively (Sullivan and Beltz, 2005). Interestingly, the first generation of neuronal precursors migrate away from the niche after each cellular division, suggesting that niche cells are not self-renewing and that the niche is supplied by another cellular source. Several studies have shown, by different techniques, a close relationship between the neurogenic niche and the vascular system/blood cells: 1) dye-conjugated dextran injections within the dorsal artery showed that the neurogenic niche lies on a complex vasculature network (Sullivan *et al*.,

2007; Sandeman *et al*., 2009); 2) the vasculature is connected to the niche beginning in the early stages of development (Sintoni *et al*., 2012); and 3) the mature brain is surrounded by blood vessels, as observed in semi-thin sections (Zhang *et al*., 2009), and blood cells are frequently found in the connective tissue below the neurogenic niche, showing similar morphology with one of the cell types in the niche (Chaves da Silva *et al*., 2012). Taken together, these data reinforce the close relationship of the blood cells and vasculature with the neurogenic niche.

Blood cells are selectively attracted to the niche via the vasculature. *In vivo* experiments using blood cells (hemocytes) and cells from three different types of tissue were isolated and labeled with a fluorescent marker. They were then set on a crayfish brain culture in order to observe the affinity of these cells to the neurogenic niche. Interestingly, only hemocytes, especially semigranular cells, were significantly attracted to the niche, particularly to the vascular cavity (Benton *et al*., 2011). Semigranular cells are derived from a precursor cell named type I, the hematopoietic stem cell (Lin and Söderhäll, 2011). Recently, Benton *et al*. (2014) have also shown that hemocytes are able to invade the neurogenic niche and that their descendants are able to differentiate into neurons. This suggests that the crustacean brain has an "open" niche which is populated by blood-born cells, and that they are able to differentiate into neural progenitors. These studies reinforce the classical view that stem cells possess greater differentiation potential than previously thought, as we stated above for vertebrates. In crustaceans as well, it seems that the ectodermal origin of embryonic neural tissues is not the only source of neurons in the adults.

A morphological study, using transmission electron microscopy, also demonstrated a specific cell type in the niche, which is by far the most numerous cell type, and that shows characteristics which suggest a role in regulating transport from the blood into the niche cells. It lines a vascular cavity located in the center of the niche, which is confluent with the vascular system of the animal (Chaves da Silva *et al*., 2012). Through different lines of reasoning, studies in the crayfish *P. clarkii* have led to the idea that cells emerging from the hematopoietic system, and circulating in the hemolymph may reach the neurogenic niche and transform into neuronal precursor cells (Zhang *et al*., 2009; Beltz *et al*., 2011; Sintoni *et al*., 2012; Chaves da Silva *et al*., 2012). The reasoning is as follows: 1) ablation of the hematopoietic tissue results in a decrease of proliferating cells within the neurogenic niche (Benton *et al*., 2014); 2) cells that surround the niche show similar morphology to cells of the APC (Chaves da Silva *et al*., 2013); 3) blood vessels, perivascular cells and hemocytes have the capacity to penetrate the tissue surrounding the neurogenic niche (Zhang *et al*., 2009; Benton, 2011; Chaves da Silva *et al*., 2012, 2013). These data suggest that blood stem cells may be a cellular source to supply the niche and also to ‘‘transdifferentiate’’ into a neural progenitor (for a review, see Hartenstein, 2014).

Regeneration and repair in invertebrates

Many animals are able to regenerate at least to some degree, as a strategy to maintain form and function throughout life. Many studies, closely linked with stem-cell research, have been conducted in different animal models, and have provided some of the most promising information to advance regenerative medicine. However, not all animal tissues have the same capacity to regenerate. The extreme example of loss of the regenerative function is the most complex and intriguing system of the body, the nervous system (Tanaka and Ferretti, 2009; Bonfanti, 2011). Some representative published data on the use of different invertebrate models for regeneration are shown in Table 1.

In metazoans in general, the capacity of the CNS to regenerate decreases in parallel with an increase in complexity (Brockes and Kumar, 2008). However, tunicates, the group to which ascidians, the sister group of vertebrates, belong, have a high CNS regenerative capacity. The first complete study of the regeneration of their brain used *Ciona intestinalis* as the experimental model (Mingazzini, 1891). The CNS was ablated and the recovery of neural components was observed. This recovery is considered a unique phenomenon among chordates (Jeffery, 2015). Experiments using the same method of mechanical ablation of the cerebral ganglion of *C. intestinalis* showed that the entire CNS can be regenerated within a few weeks (Bollner *et al*., 1997; Dahlberg *et al*., 2009). Bollner *et al*. (1997) suggested that post-mitotic cells migrate to the redeveloping ganglion cells external to the CNS, and Dahlberg *et al*. (2009) showed that neurons supply cells for reformation of the central network. Our group has recently shown that hematopoietic stem cells were present in the regenerating nervous tissue of the ascidian *S. plicata,* after neuronal damage was produced with 3-acetylpyridine (Medina *et al*., 2014).

In some groups such as annelids, the CNS is efficiently and functionally regenerated following mechanical trauma (Baylor and Nicholls, 1971; Jansen and Nicholls, 1972), and the attraction of microglia/macrophages is a key step in the engagement of an adaptive response leading to axonal sprouting. This may suggest that an optimal regeneration requires microglia/macrophages for initiation of CNS regeneration (Baylor and Nicholls, 1971). Interestingly, regeneration in the leech *Hirudo medicinalis* (phylum Annelida) proved to remain possible when glial cells were destroyed by intracellular injection of protease (Elliott and Muller, 1983). In this context, we can ask what other cell types might be involved in the regeneration of the leech CNS. In the leech, it has been suggested that blood cells are important to facilitate and accelerate the process of regeneration (Boidin-Wichlacz *et al*., 2012). Furthermore, circulating blood cells also appeared capable of infiltrating the injured CNS where, in conjunction with microglia, they limited the formation of a scar (Boidin-Wichlacz *et al*., 2012). In contrast, in mammals, CNS injury leads to the generation of a glial scar that blocks the mechanism of regeneration by preventing axonal regrowth (Fitch and Silver, 2008).

Table 1 Some representative published data on the use of different invertebrate models for regeneration and neurogenesis

Table 1. Some representative published data on the use of different invertebrate models for regeneration and neurogenesis.

Animal model	*Study focus*	*General regenerative/ hematopoietic process*	*References*
Leech *(Hirudo medicinalis)*	Regeneration	Leech blood optimized CNS neural repair through the release of neurotrophic substances	*Boidin-Wichlacz et al., 2012*
Leech (not shown)	Regeneration	Regeneration can occur between individual nerve cells in the CNS of a leech with a high degree of specificity	*Jansen and Nicholls, 1972*
House cricket (*Acheta domesticus*)	Neurogenesis	Undifferentiated cells persisted, divided and gave rise to cortical interneurons during adult life.	*Cayre et al., 1994*
Crayfish *(Procambarus clarkii)*	Neurogenesis	Ultrastructure features of the neurogenic niche and its close relationship with the vascular tissue/blood cells were shown	*Chaves da Silva et al., 2012*
Crayfish *(Procambarus clarkii)*	Hematopoietic cells (HPT)/ Neurogenesis	Cells from the anterior proliferation center (APC) showed characteristics of multipotent stem cells and had direct access to regions in the central nervous system	*Chaves da Silva et al., 2013*
Crayfish *(Procambarus clarkii)*	HPT/ Neurogenesis	There is a physical link between the HPT and the brain: APC. APC cells rapidly divide and form cell clusters *in vivo*.	*Noonin et al., 2012*
Crayfish *(Procambarus clarkii)*	Neurogenesis	Niche cells have symmetrical divisions and both daughters migrate to differentiate into neurons. Additionally, there is an association between vascular cells and niche cells.	Zhang *et al.*, 2009
Ascidian *(Botrylloides violaceus)*	Whole-body regeneration (WBR)	Multiple stem cell types underwent proliferation in the peripheral vasculature before differentiating into epithelial tissues of all three germ layers during WBR	*Brown et al., 2009*
Ascidian *(Ciona intestinalis)*	Regeneration	After the ganglion ablation, the new neural cells were formed from a "blastema" (a regenerative unit composed of a mass of undifferentiated cells)	*Dahlberg et al., 2009*
Ascidian *(Styela plicata)*	Regeneration	The success of neuroregeneration was shown to depend on the recruitment of hematopoietic stem cells and their interaction with neurons and glial cells.	*Medina et al., 2009*
Ascidian *(Ciona intestinalis)*	Regeneration	Sub-population of new neurons derived from GnRH-li neuroblasts were born prior to ablation and migrated to the regenerating ganglion.	*Bollner et al., 1997*

As noted above, a hematopoietic site was found to be correlated with adult neurogenesis in crustaceans (Noonin *et al*., 2012). However, although neurogenesis is associated with regeneration in adult animals, to better comprehend regeneration in crustaceans, the first step is to understand the cellular and molecular basis of nerve-fiber degeneration. We have addressed this issue by characterizing cellular and biochemical strategies peculiar to neurodegeneration in crustaceans (Corrêa *et al*., 2005; Chaves da Silva *et al*., 2010, 2013). Immune cellular features, such as: 1) the recruitment of granulocytes, and secondarily, of hyalinocytes to the lesion site; 2) the attraction of

a larger number of cells 48 h after the lesion (subacute phase) than 24 h after the lesion (acute phase); and 3) the presence of activated glial cells, revealed with microglia/macrophage markers, suggest that molecules released from granulocytes in the acute phase attract hyalinocytes, thus producing more undifferentiated cells that are able to differentiate into either mature blood cells or even other lineages (Chaves da Silva *et al*., 2010, 2013). According to Hartenstein (2006), hemocyte progenitors are rarely released into circulation under normal conditions, but they can appear under pathological conditions, such as injuries and pathogen invasion.

In conclusion, we suggest that the blood-lineage cells are associated not only with the functions that are usually attributed to them, but are the cells that produce neuroactive substances that induce other cell types to differentiate into neural cells. Another possibility is that the blood-lineage cells are those cells that eventually differentiate into neural cells. Figure 3 summarizes what is currently known regarding the role of hematopoietic stem cells in a crustacean during adult neurogenesis and in an ascidian during brain repair.

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