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SPOTLIGHT

Non-traditional roles of immune cells in regeneration: an evolutionary perspective

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ABSTRACT

Immune cells are known to engage in pathogen defense. However, emerging research has revealed additional roles for immune cells, which are independent of their function in the immune response. Here, we underscore the ability of cells outside of the adaptive immune system to respond to recurring infections through the lens of evolution and cellular memory. With this in mind, we then discuss the bidirectional crosstalk between the immune cells and stem cells and present examples where these interactions regulate tissue repair and regeneration. We conclude by suggesting that comprehensive analyses of the immune system may enable biomedical applications in stem cell biology and regenerative medicine.

KEY WORDS: Stem cells, Regeneration, Innate immunity, Trained immunity, Macrophages, Hemocytes, Homeostasis

Introduction

The immune system is traditionally viewed as a defense against infection (Nicholson, 2016). Research from the past 20 years, and particularly more recently, has revealed that immune cells are involved in more than just the fight against germs. New and exciting interactions, such as crosstalk between stem cells and immune cells, are being explored. Such interactions are observed in the development, homeostasis, or post-inflammatory regeneration of tissues across evolution. Here, we describe the evolution of adaptive immune-like responses (trained immunity) in cell types outside of the adaptive immune system. We then tackle the reverse observation: how immune cells perform non-immune functions related to regeneration and stem cell regulation. We hope to convey a broader consideration of the types of immune responses and non-immune functions of traditional immune cells in the context of regeneration and evolution.

Evolution of trained immunity

Host immunity is typically divided into innate and adaptive components. The innate immune system includes the activity of myeloid cells, such as macrophages, that react quickly to infection with non-specific responses, such as phagocytosis (Wynn and Vannella, 2016). When the innate immune response is insufficient to clear the infection, the adaptive immune system generates an antigen-specific response, as well as long-term immunological memory that allows rapid response upon reinfection of the antigen (Farber et al., 2016). Because adaptive immunity is a recent evolutionary trait, likely originating from jawless vertebrates (Pancer et al., 2004; Boehm and Swann, 2014), most studies on immune memory focus on recently evolved animals. In evolutionary terms, this would imply that no adaptive or memory-like traits would have existed before jawless vertebrates, nor would they exist in organisms with only innate immunity. This is an immunological fallacy. There is ample evidence of innate immune memory occurring from plants and invertebrates to innate immune cells in mammals (Pradeu and Du Pasquier, 2018; Netea et al., 2019).

To understand the non-immune functions of immune cells, we must consider that immunity occurs throughout evolution and that many other cells, in addition to dedicated immune cells, have forms of immunity. One example is trained immunity, which involves epigenetic mechanisms (such as DNA methylation and chromatin reorganization) to provide persistent functional reprogramming in response to a pathogenic signal. As a result, subsequent responses to recurring pathogenic signals are enacted with more (or less) vigor depending on the context (Fanucchi et al., 2021). For example, trained cells may react more passively to antigens that are consistently present in the environment but will escalate the response to a recurring pathogenic antigen. Therefore, it is important to consider that the evolution of immunity not only requires the ability to recognize foreign antigens but to distinguish between them and react appropriately. Although trained immunity to reinfection has been well-documented in plants (‘systemic acquired resistance’; Kachroo and Robin, 2013), we focus here on trained immunity in animals (Table 1).

Diploblastic species

Evidence of trained immunity has been documented in sponges (Hildemann et al., 1979), whereby transplanted sponge tissue from a donor of one species is rejected by the host from another species. Rejection occurs more rapidly when the host is exposed to the same donor tissue again later. A similar response has been seen within corals (Hildemann et al., 1977). These examples show that trained immunity concepts are likely to have been employed from the start of animal evolution and need to be explored further.

Protostomes

Within the protostome lineage, evidence for trained immunity is far-ranging. The platyhelminth *Schmidtea mediterranea* clears recurring *Staphylococcus aureus* infections much faster than the original infection through the activation of the peptidoglycan receptor *Smed-PGRP-2* (Torre et al., 2017). *Caenorhabditis elegans* has a conditioned behavior to pathogenic bacteria; the animal avoids pathogens already encountered through the activation of the Toll-like receptor (TLR) pathway (Zhang et al., 2005). *Drosophila* and mosquito (*Anopheles gambiae*) have each shown specific responses

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Table 1. Animals and specialized cells with regenerative ability and associated immune components

Species	Immune cell(s)	Stem cells (organ)	Immune-associated mediators	Mechanisms mediated by macrophage or macrophage-like cells	References
Hydra	Epithelial cells	Interstitial stem cells (whole organism)	TLRs; BMPs; NF-κB; RIG-I receptors Wnt signaling	Axial patterning; embryogenesis	Reinhardt et al., 2004; Broun et al., 2005; Bosch et al., 2009; Lange et al., 2011; Bosch, 2014; Wenger et al., 2014; Schröder and Bosch, 2016
Planaria	Reticular cells; intestinal phagocytes; neoblasts	Neoblasts (whole organism)	TFs; TLRs; TAK1/MKKK/p38; PGRP2; NF-κB RIG-I receptors; C-type lectins	Tissue regeneration; bacterial and fungal infections	Abnave et al., 2014; Peiris, et al., 2014; Arnold et al., 2016; Gao et al., 2017a,b; Pang et al., 2017; Torre et al., 2017; Maciel et al., 2019, 2020; Kangale et al., 2021
Earthworms	Coelomocytes	Cerebral ganglia (brain)	Riboflavin	Neurogenesis	Molnar et al., 2015
Crayfish	Hemocytes	Neuronal precursors (nervous system)	TFs	Neurogenesis	Benton et al., 2014
Mollusks	Hemocytes	Neuronal precursors (blastema)	LPS; TFs (e.g. SOX2)	Embryonic development; stem cell pluripotency; tissue homeostasis	Zhang and Cui, 2014
Drosophila	Hemocytes	Unspecified mutant stem cells (imaginal discs)	TLRs	Cell competition	Meyer et al., 2014; Alpar et al., 2018
		Müller glia (retina)	Manf	Regeneration; retinal repair	Neves et al., 2016
		ISCs (mid-gut)	Dpp	Proliferation; maintenance of basal stem cell activity	Ayyaz et al., 2015
		GSCs (ovaries)	Dpp	Assembly; homeostasis	Chen and McKearin, 2003; Van De Bor et al., 2015
		Glial cells (nervous system)	Drpr	Proliferation	Losada-Pérez et al., 2021
Salamander	Macrophages	Blastema cells (limbs); myeloid cells (retina)	TGFβ1	Vascularization; regeneration	Henry and Tsonis, 2010; Godwin et al., 2013; Zhao et al., 2016; Arenas Gómez et al., 2020
Zebrafish	Macrophages	Glial cells (retina); blastema cells (caudal fin)	TFs	Dedifferentiation; proliferation; regeneration kinetics; patterning, branching and bone quality	Wan and Goldman, 2016; Petrie et al., 2014
Mammals	Macrophages	MSCs	LPS; LIF; OSM	Bone marrow egression; osteogenesis	Saldaña et al., 2019
		ISCs	TFs	Paneth cell differentiation	Sehgal et al., 2018
		MaSCs	TFs	Developing ductal structures and adipose stroma	Gyorki et al., 2009
		HSCs	TNFα; OSM; TLRs	Proliferation	Baldridge et al., 2011
		NSCs	Remyelination cytokines; VEGF; IL10; TGFβ	Oligodendrocyte production; differentiation	Chamberlain et al., 2016

BMP, bone morphogenic protein; Dpp, Decapentaplegic; Drpr, Draper; GSCs, germline stem cells; HSCs, hematopoietic stem cells; IL10, interleukin 10; ISCs, intestinal stem cells; LIF, leukemia inhibitory factor; LPS, lipopolysaccharides; Manf, Mesencephalic astrocyte-derived neurotrophic factor; MaSCs, mammary stem cells; MSCs, mesenchymal stem cells; NF-κB, nuclear factor κB; OSM, oncostatin M; PGRP2, peptidoglycan-recognition protein; TFs, transcription factors; TGFβ, transforming growth factor beta; TLRs, Toll-like receptors; TNFα, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

to secondary infection, primarily through the action of hemocytes (Sadd and Schmid-Hempel, 2006; Rodrigues et al., 2010). These same insects also diversify their antigen receptors in response to recurring infection (Watson et al., 2005; Dong et al., 2006). Combined, the evidence for trained immunity within invertebrates is growing and requires further scientific exploration.

Deuterostomes

Echinoderms somatically recombine antigen receptors to diversify their response to recurring infections (Pancer, 2000) and other early-branching bilaterian deuterostomes also display such traits. In *Ciona intestinalis*, for example, injection of erythrocytes derived from humans or ducks leads to encapsulation of the cells upon initial exposure, which induces the activation of morula cells within the animals that quickly encapsulate the cells upon secondary infection (Wright and Cooper, 1975). Jawless lampreys were among the first animals in which trained immunity to recurring infection was appreciated. Lampreys employ recombination of variable lymphocyte receptors (VLRs), diversity of which is generated by alteration of leucine-rich repeats (Pancer et al., 2004), to produce an antigen-specific antibody response upon secondary infection (Finstad and Good, 1964). Such recombination events have become common among jawed vertebrates, although the mechanism changed radically from VLR-based to immunoglobulin (Ig)-based diversity, which is observed in fish, amphibians and amniotes (Gourbal et al., 2018; reviewed by Buckley and Dooley, 2022).

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Box 1. The immune system's role in cell competition

Cell competition is an evolutionarily conserved physiological process that eliminates undesirable cells from tissues (Morata, 2021). Although cell competition was discovered in *Drosophila*, it is also present in vertebrate animals and appears to play central roles in embryonic development, tissue maintenance, and even cancer (Baker, 2020). Thus, cell competition can be considered as an endogenous surveillance mechanism whereby unfit or outcompeted cells are eliminated based on different patterns of signaling, gene expression and lower growth rates. The elimination of the unfit cells may involve different mechanisms, such as apoptosis and epithelial extrusion (Morata, 2021). Recent research using *Drosophila* implies that surveillance through activation of the Toll pathway may lead to apoptosis of the unfit cells (Meyer et al., 2014; Alpar et al., 2018). This finding is strongly supported by Toll pathway inactivation, which reduces the elimination of unfit cells (Alpar et al., 2018). These intriguing results suggest that components of the innate immune system could be diverted to homeostatic purposes independently of infection and opens the door for further research to identify surveillance signals allowing the identification of unfit cells. Researching these non-traditional immune functions could be useful for studies or early detection of transformed cells and collective analysis of cellular turnover.

Mammals

Innate immune cells can also be trained to recognize antigens long-term, termed recurring antigen recognition. Macrophages are responsive to the exposure of host-derived stimuli (e.g. IL1, GM-CSF, TNFα and IFNγ), leading to epigenetic reprogramming of these cells and a quicker response upon secondary exposure (Saeed et al., 2014). Natural killer cells respond to similar stimuli (e.g. LTα, LTβ, TNFα and IFNγ), which also create an immune memory in these cells (Sun et al., 2012). Although mast and dendritic cells each have the capability for an induced memory response through epigenetic reprogramming, studies addressing this phenomenon are lacking (Hamada et al., 2018). Furthermore, within the central nervous system (CNS), macrophage-derived microglia (reviewed by Kraus et al., 2021; Mehl et al., 2022) display an enhanced secondary immune response after primary exposure to either viruses or bacteria (Mariani and Kielian, 2009; Haley et al., 2019). Interestingly, hematopoietic stem cells (HSCs), which can epigenetically reprogram in response to antigen themselves, can also transfer memory responses to their progeny (Baldridge et al., 2011; Kaufmann et al., 2018), indicating that all HSC-derived innate immune cells are capable of immunological memory.

Trained immunity also occurs in cells outside of the mammalian innate immune system (Hamada et al., 2018). For example, epithelial barrier tissues, formed by parenchymal stem cells, directly respond to environmental exposures immediately and in preparation for future exposure. Once differentiated, these cells are long-term residents of barrier tissues whose 'memories' can be stored longer than that of innate immune cells (Ordovas-Montanes et al., 2020). Along with fibroblasts, this epithelial barrier constitutes the first line of innate immune defense. In mice, primed skin epithelial stem cells respond quicker to wound healing upon a secondary challenge (Naik et al., 2017). Epithelial stem cell memory is long-lived because they turn over on average about once every 2 years (Tunn et al., 1989). Mesenchymal stem cells (MSCs), which can differentiate into osteoblasts, chondrocytes, myocytes and adipocytes, also have trained immunity properties. MSCs produce pro-inflammatory cytokines [e.g. IL8, MCP-1 (also known as CCL2) and IL6] upon secondary exposure to bacterial lipopolysaccharides (LPS) or eukaryotic tumor necrosis factor (TNF) (Liu et al., 2016). Although MSCs are short-lived, MSC-derived tissue-resident cells can survive for years until replaced (Eggenhofer et al., 2014). Stromal cells also exhibit immunological memory (Crowley et al., 2018); secondary exposure to *Salmonella typhimurium* causes a more rapid response owing to epigenetic modifications (Owens et al., 2013). Similarly, endothelial cells are primed by cytokines to produce IL8 upon secondary exposure (Wolff et al., 1998). Furthermore, stem cells can display immune properties and can be responsible for the generation of immune memory in their progeny, including cells that are not traditionally known for their immune function (Eisenhoffer et al., 2008; Torre et al., 2017). Collectively, innate immune cells and non-immune cells are capable of immune memory, although more research is required.

The non-immune functions of immune cells in relation to stem cells and regeneration

Increasing evidence shows that immune cells actively and passively interact with stem cells to regulate stem cell behavior (e.g. cell cycle, differentiation and migration) during development and regeneration (Zhang, 2012; Chung and Son, 2014; Wynn and Vannella, 2016; Abnave and Ghigo, 2019; Arenas Gómez et al., 2020; Ballarin et al., 2021). Below, we describe examples in which the immune cells from various organisms interact and modulate other tissues during regeneration. Other non-immunological functions of the immune system, such as cell competition (Box 1), also appear to be mediated by macrophage-like cells.

Mammalian macrophages

Whether the response is local or systemic, macrophages are one of the main effectors of the innate immune response. Macrophages phagocytose pathogens, foreign bodies, dead diseased/tumor cells and/or debris during tissue remodeling (Hirayama et al., 2017). They also perform recruitment and regulatory functions by secreting a range of molecules, chemokines, cytokines and growth factors, which facilitate cellular crosstalk outside of the immune system. Macrophage function and interactions are wide-ranging because different subpopulations exist, depending on the environment, lineage and/or organism (Takahashi et al., 1996; Italiani and Boraschi, 2014). For example, in mammals, the heterogeneity of macrophages can be classed by macrophage location (circulating versus tissue-resident macrophages; reviewed by Cox et al., 2021; Lee and Ginhoux, 2022), polarization state (anti-inflammatory versus pro-inflammatory macrophages versus regulatory macrophages), or developmental origin (Fig. 1) (Williams et al., 2018; Qian et al., 2019). Macrophages interact with stem cells via a variety of factors. We, therefore, suggest that a holistic view of the immune system could reveal that macrophages and other immune components are crucial for regeneration and development (Fig. 2).

Intestine

Immune responses in the mammalian intestines have to be tolerant of commensal microbiota and also adequately respond to any harmful pathogens or injury (Macdonald and Monteleone, 2005). Close and continuous interaction between immune cells and molecules with intestinal stem cells (ISCs) is therefore important for regeneration (Mowat and Agace, 2014; Hou et al., 2020). Studies by Biton and colleagues have shown crosstalk between ISCs and immune cells via cytokines, such as IL10, IL13 and IFN, to influence the renewal, differentiation and fate of ISCs (Biton et al., 2018). Additionally, leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5)-expressing ISCs act as unique

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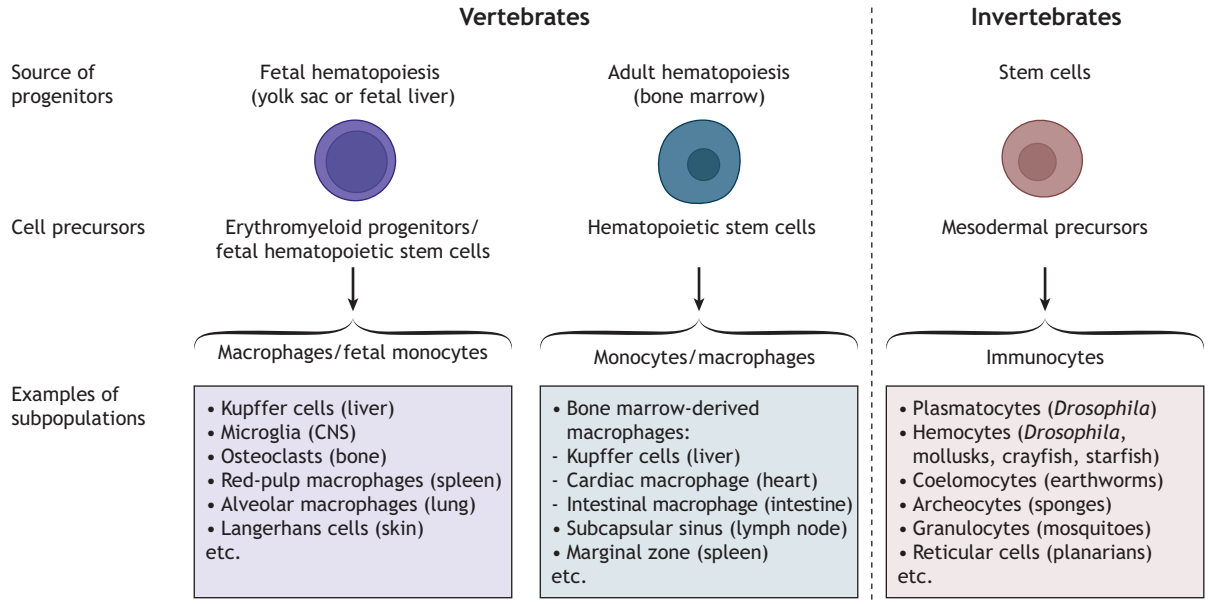

Fig. 1. Macrophages and macrophage-like cells exist in different forms, states and subpopulations in both vertebrates and invertebrates. Because of their heterogeneous nature and plasticity, macrophages have a wide range of functions, which might include unique roles specific to their environment. Macrophages in vertebrates originate from the yolk sac and fetal liver during fetal hematopoiesis or the bone marrow during adult hematopoiesis, whereas macrophage-like cells originate from the hematopoietic organs/tissues in invertebrates. Some known examples of macrophages in both vertebrates and invertebrates are shown. Created with BioRender.com.

antigen-presenting cells when interacting with T helper cells, and this interaction impacts epithelial cell differentiation and fate (Sato et al., 2011; Biton et al., 2018). Macrophages are also involved in maintaining this balance; for example, colony-stimulating factor 1 (CSF1)-dependent macrophages regulate intestinal epithelium differentiation; depletion of CSF1R-dependent macrophages affects the differentiation of intestinal epithelial cell lineages, such as Paneth cells, and depletes $Lgr5^{+}$ ISCs (Sehgal et al., 2018). Thus, several immune cells (such as T cells, innate lymphoid cells, dendritic cells and macrophages), as well as cytokines secreted from these cells, regulate ISCs and are therefore responsible for regenerative functions in the gut.

Mammary gland

CSF1 recruits monocytes to the terminal end buds of the mammary glands and promotes monocyte-to-macrophage differentiation (Van Nguyen and Pollard, 2002). Mammary stem cells (MaSCs) also require these macrophages to maintain the stem cell microenvironment (Gouon-Evans et al., 2002); in the absence of these macrophages, the activity and replenishment of the MaSC population are reduced. Morphological defects in the development of mammary ducts, potentially due to negative impacts on putative progenitor cells, also occur when macrophages are absent (Gyorki et al., 2009; Wang et al., 2020).

Nervous system

In the nervous system, activation of the proinflammatory response impacts neural stem cell behavior to promote oligodendrocyte production (Carpentier and Palmer, 2009). Recruited macrophages secrete pro-myelination cytokines that drive re-myelination and promote oligodendrocyte differentiation (Kotter et al., 2005; Chamberlain et al., 2016). Conversely, proinflammatory cytokines [e.g. IL6 and transforming growth factor beta (TGFβ)] impair neuroblast proliferation and neurogenesis when chronically over-produced (Aarum et al., 2003; Carpentier and Palmer, 2009). Upon nervous system injury, M2 macrophages produce cytokines (e.g. IL10 and TGFβ), which induce cell proliferation and angiogenesis to promote tissue regeneration. M2 (polarized) macrophages also secrete vascular endothelial growth factor (VEGF), which maintains endothelial cells necessary for angiogenesis and provides nutrition for nerve tissue repair (Liu et al., 2019). Additionally, increasing the ratio of M2/M1 macrophages promotes nerve repair and increases the number of regenerated axons (Armstrong et al., 2003). Thus, macrophages may instruct integral repair processes of the nervous system.

Blood, bone and the cardiovascular system

The immune system also affects hematopoiesis. TNFα, a common pro-inflammatory cytokine, regulates HSC proliferation (Baldridge et al., 2011). In addition, interferon gamma (IFNγ) directs progenitors towards myeloid differentiation, and chronic IFNγ stimulation leads to HSC exhaustion (Orford and Scadden, 2008; Baldridge et al., 2010, 2011). Furthermore, a unique CD169 $(SIGLEC1)^{+}$ macrophage population is responsible for HSC retention in the bone marrow and for the ability to discriminate non-self (Chow et al., 2011). In cardiovascular conditions (such as atherosclerosis) macrophages produce TNFα and oncostatin M (OSM), which cause vascular calcification (Guihard et al., 2012). Osteoclasts, tissue-resident macrophages of the bone, also regulate aspects of bone maintenance and formation, especially during inflammatory events and bone injury (reviewed by Yahara et al., 2022).

Mesenchyme

MSCs are active participants in the wound-healing phase of inflammation (Freytes et al., 2013). MSCs are activated by the pro-inflammatory signals from monocytes and in turn produce growth factors [e.g. VEGF and platelet-derived growth factor (PDGF)], causing the recruitment of stromal cells, endothelial cells, stem cells and others (Freytes et al., 2013). In a cyclin-dependent

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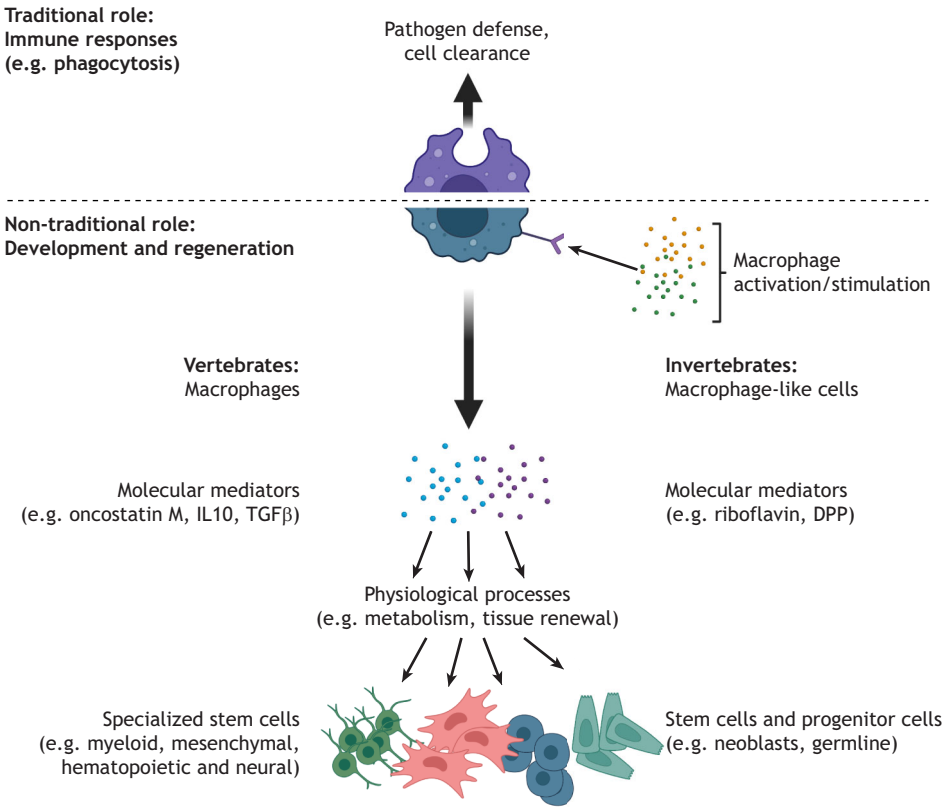

Fig. 2. Traditional and non-traditional roles of the immune system. The traditional role of the immune system is to provide defense against pathogens. Highlighted are some of the immune cells (macrophages), immune-associated molecules, and the various stem cells with which they interact in vertebrates (left) and invertebrates (right). Current interpretations of the immune system include non-immune functions and interactions with other cells (such as stem cells) to modulate physiological functions, such as tissue regeneration and repair. Created with BioRender.com.

mechanism, the MSCs, in turn, promote monocytes and macrophages to egress from the bone marrow (Chung and Son, 2014). This crosstalk between MSCs and macrophages is important in tissue repair.

Macrophages in anamniotes

Interplay between macrophages and regeneration also exists in other vertebrate models. For example, macrophages are involved at different stages of adult zebrafish tail fin regeneration. During the initial stages of tissue regrowth, *mpeg1*$^{+}$ macrophages influence the proliferative capacity of the blastema, as well as the length, patterning, branching and bone quality of the caudal fin (Petrie et al., 2014). Additionally, macrophage-derived TNFα controls blastemal cell proliferation during larvae fin remodeling (Nguyen-Chi et al., 2017). In the zebrafish retina, glia cells dedifferentiate to a stem cell-like state, thereby inducing retinal regeneration and repair (reviewed by Becker and Becker, 2022). Moreover, macrophages promote glia or progenitor proliferation and regulate rod cell regeneration kinetics (Wan and Goldman, 2016; White et al., 2017).

In salamander limb regeneration, the early depletion of macrophages causes wound closure and prevents regeneration (Godwin et al., 2013; Zhao et al., 2016). However, macrophage depletion after blastema formation slows limb growth and reduces surface vasculature (Godwin et al., 2013; Zhao et al., 2016; Bolaños-Castro et al., 2021). In the absence of macrophages, the expression of TGFβ1 and limb regeneration is also negatively affected (Lévesque et al., 2007). During lens regeneration in both newts and salamanders, the myeloid cell recruitment to initiate regeneration is macrophage dependent (Henry and Tsonis, 2010). Furthermore, in zebrafish and amphibians, similar roles of macrophage involvement have been observed, highlighting that macrophages are a conserved regulatory factor of regeneration.

Invertebrate immunocytes

In invertebrates, phagocytic and effector roles are also performed by macrophage-like cells or primitive macrophages, sometimes referred to as ‘immunocytes’ (Ottaviani and Franceschi, 1997), that encompass hemocytes, coelomocytes, plasmatocytes and granulocytes, as well as all other cells that serve an equivalent function of vertebrate macrophages (Lavine and Strand, 2002; Abnave et al., 2017) (Fig. 1). Although immunocytes differ in their appearance and location depending on the species, they mediate innate immune responses in many invertebrate organisms (Franchini et al., 1996; Ottaviani, 2011; Abnave et al., 2017). In understanding aspects of the invertebrate immune system, it is important to recognize that there can be additional noncanonical functions, such as modulation of tissue renewal and regeneration.

Lophotrochozoa

Beyond their traditional immune functions, immunocytes influence stem cell proliferation and differentiation. In earthworms (*Dendrobaena veneta*), immune-competent coelomocytes play an active role in adult brain regeneration; brain tissue renewal is reduced after the depletion of coelomocytes and coelomocyte-associated molecules, such as riboflavin (Molnar et al., 2015). In mollusks (e.g. *Anodonta woodiana*), bacterial LPS stimulates a pro-inflammatory response involving the production of transcription factors, such as SRY-box transcription factor 2 (SOX2), which are essential in embryonic development, stem cell pluripotency and tissue homeostasis (Zhang and Cui, 2014; Xia et al., 2019).

Arthropods

In crayfish, in which neuronal precursors do not self-renew, hemocytes appear to serve as precursors in adult brain neurogenesis (Benton et al., 2014). In *Drosophila*, the cellular immune response is

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mediated by hemocytes that primarily serve immune functions, but also interact with other tissues to modulate cellular decisions and tissue repair. Hemocytes secrete Decapentaplegic (Dpp), a TGFβ/BMP homolog, which induces intestinal stem cell proliferation and maintains basal stem cell activity (Ayyaz et al., 2015; Chakrabarti et al., 2016). Local ovarian hemocytes in *Drosophila* regulate the assembly and homeostasis of germline stem cells (GSCs) because Dpp overexpression disrupts germ cell differentiation (Xie and Spradling, 1998). In addition to Dpp, loss of hemocyte-derived collagen IV during gonad development alters GSC niche organization, indicating that hemocytes are also involved in ovarian tissue morphogenesis (Chen and McKearin, 2003; Van De Bor et al., 2015). Moreover, *Drosophila* retina regeneration is promoted by the hemocyte-derived anti-inflammatory factor mesencephalic astrocyte-derived neurotrophic factor (Manf) (Neves et al., 2016). During wing development and maturation, hemocytes secrete extracellular matrix (ECM) to join the dorsal and ventral wing blades (Kiger et al., 2001; Gold and Brückner, 2015). Hemocytes are also recruited to injury sites following CNS trauma, where they stimulate glial cell proliferation and participate in neural functional recovery (Losada-Pérez et al., 2021). Therefore, hemocytes in *Drosophila*, whether circulating or tissue-resident, facilitate some regenerative processes beyond their expected immunoregulatory functions.

Planarians

Flatworm planarians can regenerate the entire body from small tissue segments, owing to an abundance of stem cells (neoblasts). Therefore, it seems logical that stem cells and immune cells engage in an orchestrated process to avoid infection of the open wound while regeneration occurs. The planarian immune response has recently attracted attention because of its antimicrobial properties, which effectively and rapidly clear infection (Abnave et al., 2014; Pang et al., 2016; 2017; Gao et al., 2017a,b; Maciel et al., 2019). This response is achieved by the activation of an evolutionarily conserved innate immune system, capable of clearing pathogenic infections that can be lethal to humans (e.g. *Mycobacterium tuberculosis*, etc.) (Abnave et al., 2014; Peiris et al., 2014; Pang et al., 2016; Gao et al., 2017a,b; Maciel et al., 2019, 2020; Kangale et al., 2021). In addition, the ability to study the whole-body host response to infection provides unique opportunities to obtain comprehensive insights into immune system interactions with other tissues. For example, fungal infection of planarians with *Candida albicans* leads to neoblast hyperproliferation, transcriptional expression of neural genes and increased mucus secretion (Maciel et al., 2019, 2020), suggesting that fungal infection is followed by an integrated response that includes the immune cells, neoblasts and differentiated tissues. Although the sequential order of activation and molecular regulation of this response requires further investigation, evidence in planarians suggests that the immune system cooperates with other cells to enable systemic clearance of fungal pathogens. Furthermore, recent studies analyzing the endogenous microbiota in the planarian *S. mediterranea* have revealed that the innate immune system influences regeneration (Arnold et al., 2016). Activation of the TAK1/MKKK/p38 signaling pathway can either enhance or repress cell death during infection or regeneration, respectively (Arnold et al., 2016). Although how the same signaling pathway drives cell fate in different directions remains poorly understood, these results indicate that the planarian innate immune system can differentiate between infection and tissue repair. Other studies have identified intestinal planarian phagocytes expressing the transcription factor-like nuclear factor κB (NF-κB), lack of which causes intestinal tissue lysis when it is knocked down with RNAi (Forsthoefel et al., 2012). Together, the evidence in planarians suggests that the immune system not only clears infections but interacts with other tissues to regulate cellular fate decisions essential for tissue maintenance and repair.

Cnidarians

Like planarian, *Hydra* regenerates damaged tissues from a robust population of stem cells. Injury or infection of the *Hydra* results in the removal of infected cells by apoptosis or phagocytosis, induction of antimicrobial peptides (AMPs), wound closure by epithelial cells and reconstruction of the ECM (Reddy et al., 2019). During tissue restoration after injury or infection, immune and regenerative processes are both implemented (Shimizu et al., 2008). However, ongoing work is establishing the molecular and genetic components of these processes. Genetic studies have revealed an upregulation of genes and pathways responsible for phagocytosis, cell death-like MAP kinase signaling and Jun N-terminal kinase (JNK) signaling (Petersen et al., 2015). Interestingly, forkhead box O (FoxO), a transcription factor that drives stem cell renewal, is also involved in the stress response in *Hydra* (Bridge et al., 2010; Boehm et al., 2012). Under stress, FoxO activity is increased via the JNK pathway, which subsequently affects AMP expression (Boehm et al., 2012). Silencing of FoxO expression through shRNAs reduces AMPs expressed in the *Hydra*, establishing a link between stress, stem cells and immunity (Boehm et al., 2012). More research should be devoted to studying the interplay between *Hydra* immunity and regeneration.

Conclusion

The immune system is traditionally studied as a dynamic and highly adaptable physiological response to fight pathogens. Here, we draw attention to the notion that immune cells actively and passively influence the function of many tissues, well beyond their traditional roles in immunity. The mechanisms by which the immune system engages with other tissues are diverse and are only beginning to be uncovered. Macrophages appear to be one of the most versatile immune cells, driving bidirectional crosstalk with non-immune cells, including stem and progenitor cells. From the evolutionary perspective, stem cells and other non-adaptive immune cells have non-traditional roles in immunity. They display traits of immune memory and, thus, cells that are not commonly associated with the immune system may fight recurring infections. Overall, the non-traditional aspects of the immune system are an essential component for embryonic development, tissue homeostasis and repair. As such, we advocate for renewed attention to comprehensive analysis underscoring the natural interactions of tissues at the organismal level. These types of studies across model organisms would be instrumental to unleash biomedical advances in different fields encompassing immunotherapy, neuroimmunomodulation and regenerative medicine.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: B.N.A., Y.A.I., J.A.G., N.J.O.; Writing – original draft: B.N.A., Y.A.I., J.A.G., N.J.O.; Writing – review & editing: B.N.A., Y.A.I., J.A.G., N.J.O.

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