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Crustacean hematopoiesis

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ABSTRACT

Crustacean hemocytes are important mediators of immune reactions, and the regulation of hemocyte homeostasis is of utmost importance for the health of these animals. This review discusses the current knowledge on the lineages, synthesis and differentiation of hemocytes in crustaceans. Hematopoietic tissues, their origins, and the regulation of hematopoiesis during molting, seasonal variation and infection are discussed. Furthermore, studies concerning the molecular regulation of hemocyte formation in crustaceans are also described, and the different lineages and their molecular markers are discussed and compared with several insect species. Signaling pathways and the regulation of hematopoiesis by transcription factors are typically conserved among these arthropods, whereas cytokines and growth factors are more variable and species specific. However, considering the great diversity among the crustaceans, one should be cautious in drawing general conclusions from studies of only a few species.

1. Introduction

Crustaceans form an extremely diverse group, comprising between 40,000 and 60,000 species, and constitute a large proportion of the biomass in the oceans (Giribet and Edgecombe, 2012). The blood cells, or hemocytes, of crustaceans are important players in immune reactions, and these cells contain important immune proteins, such as the components of the prophenoloxidase-activating system (proPO) in the melanization cascade, and they can perform cellular reactions, such as the phagocytosis of small foreign objects or encapsulation of larger intruders. A large number of original studies regarding crustaceans and invertebrate innate immunity in general have been conducted in the freshwater crayfish *Pacifastacus leniusculus*; among their findings, the proteolytic cascade that controls melanization is one of the most important (Cerenius et al., 2010, 2008; Jiravanichpaisal et al., 2006). The proPO system resides primarily in granular cells (GCs). These and other hemocytes are produced throughout the life of the animal in a specific hemocyte-forming tissue called hematopoietic tissue (HPT) (Lin and Söderhäll, 2011). When a parasite or foreign object enters the hemocoel (body cavity), certain hemocyte types perform phagocytosis and encapsulate these objects. Because hemocytes are consumed in these processes, there is a need for rapid synthesis of several new hemocytes during infection. However, the production of hemocytes during normal non-infectious conditions is also needed. Freshwater crayfish belong to the decapods, and among the Crustacea, this order is the most studied with regard to immunology and hematopoiesis. Indeed, only a few species from other groups have been investigated, and none in great detail. Previous studies concerning hematopoiesis in crustaceans other than decapods are summarized in Table 1 (phylogeny simplified from Meland and Willassen, 2007). However, it is noteworthy that the phylogenetic relationships among different crustacean types are controversial and have been revised several times during the past decades; the difficulties in robustly clarifying ancient relationships within the crustaceans have been discussed in detail (Rota-Stabelli et al., 2013). Thus, the aim of the following review is to provide an overview of the current knowledge about hematopoiesis in crustaceans. Notably, there is significant diversity among the species in the crustacean subphylum, and the limited studies have mainly focused on the economically important decapods. Therefore, general conclusions concerning the entire decapod group should be drawn with great care because studies in a few species may not represent the extensive collection of available crustacean species.

2. Structure of the circulatory system

According to most textbooks, crustaceans have an open circulatory system. However, recent studies have clearly shown that the

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Table 1
Studies of hematopoietic tissues in crustaceans.

Taxon	Species	HPT location	Refs.
Branchiopoda	*Artemia salina*	Bilaterally at the base of each limb	(Debaisieux, 1952; Lochhead and Lochhead, 1941)
	Chirocephalus diaphanus		(Bruntz, 1907)
	Phyllopoda	No HPT	
Leptostraca	*Nebalia geofroyi*	No HPT	(Bruntz, 1907)
Stomatopoda	*Squilla mantis*	Surface of ventral artery	(Bruntz, 1907)
Euphausiacea		No report	
Mysida	*Mysis charnaelo M. oulgaris*	Surface of cardiac stomach	(Bruntz, 1906)
Syncardia		No report	
Decapoda	*Several species*[a]	APC and HPT surrounding ophthalmic artery	(Allen, 1893)[a]
Thermosbaenacea		No report	
Spelaeogriphacea		No report	
Amphipoda	*Talitre, Gammarus*	Bilateral lobes in the head	(Bruntz, 1907)
Lophogastrida		No report	
Mictacea		No report	
Stygiomysida		No report	
Tanaidacea		No report	
Cumacea	*Iphinoë*	Bilateral lobules at the fifth thoracic segment	(Bruntz, 1907)
Isopoda	*Oniscus, Asellus, Ligia, Anceus*	Lobules in the last 2 thoracic and abdominal segments	(Bruntz, 1907)
	Armadillidium		(Bruntz, 1907)
			(Chevalier et al., 2011)

[a] After the first report by Allen in 1893, numerous studies, as presented in this review, concerning HPT in different decaopds are available.

degree of vascular branching markedly varies among species, and well-developed cardiovascular systems in decapods have occasionally been demonstrated. Thus, the crustacean circulatory system is more accurately described as "incompletely closed" (McGaw and Stillman, 2010; Reiber and McGaw, 2009). The crustacean heart is shaped like a pentagonal chamber, placed dorsally above the gills, and surrounded by a pericardium from which the hemolymph is transported through segmentally paired, laterally arranged ostia with valves into the heart. In less developed crustaceans, such as entomostracans and lower malacostracans, the heart is more primitive and is simply a long, expanded artery with ostia. During embryogenesis, the heart develops from a dorsal vessel. Hemolymph is transported from the heart anterially by non-muscular arteries that penetrate the pericardial membrane and branch into different parts of the head, ultimately delivering the hemolymph to sinuses throughout the entire body. All the sinuses are circumscribed by a membrane and lead the hemolymph to the gills via large ventral sinuses. Efferent arteries then leave the gills and bring oxygenated hemocyanin back to the heart through the ostia (Bauchau, 1981). The decapod crustaceans have a well-developed vascular system with arterioles penetrating the organs in the body (McGaw and Stillman, 2010). Although the fine arteries are blind ended, the sinuses, at least in some decapods, seem to be defined units formed as discrete but large channels delineated by fibrous connective tissue, making the circulatory system partially closed (McGaw, 2005). However, unlike vertebrates, crustaceans have no separate lymphatic system, and the main transport is provided through a single bodily fluid, the hemolymph. Therefore, the liquid in the crustacean circulatory system is not typically referred to as blood because it is a combination of blood (hemo or haemo [Latin]) and the colorless fluid of the body (lympha – water or clear water [Latin]).

In crustaceans, hemolymph comprises cells, water, and dissolved inorganic salts and proteins, among which the oxygen carrier hemocyanin is the most abundant. The free cells found in hemolymph are accordingly named hemocytes (Johnson, 1980). Thus, hemocytes are the free-moving inhabitants of the circulatory system, which circulate into the smallest arterioles in the brain and penetrate into the tissues surrounded by the sinuses, where they perform their tasks as mediators of immune defense and other functions.

3. Crustacean hemocytes

3.1. Morphology

Carus initially characterized crustacean hemocytes in the early 1800s (*Carus, C. G. Von den aussern Lebensbedingungen der weiss- und kalt-blutigen Thiere. Leipzig, 1824, pp.85–86*), and Bauchau (1981) previously reviewed this and other studies. Hemocytes are divided into different types based primarily on morphological criteria and secondarily on functional properties. Hemocyte types have been described and classified mainly for the decapods. Classifications according to morphology, ultrastructural studies, and staining properties were conducted in several species during the second half of the 1900s (Hose et al., 1990, 1987, 1987; Johansson et al., 2000; Martin et al., 1999; Toney, 1958; Vázquez et al., 1997; Wood and Visentin, 1967). Bauchau (1981) suggested a classification scheme in decapod crustaceans, dividing the cells into hyaline, semigranular and granular hemocytes, and this classification is used in the following text (Table 2, rewritten with permission from Bauchau, 1981).

Hemocytes have also been described in other crustacean species, although most studies have focused on the decapods. For example, studies in the branchiopod *Artemia* (the brine shrimp) have shown that this species only possesses granular hemocytes (Lochhead and Lochhead, 1941; Martin et al., 1999). Hemocytes in *Artemia* show similarities to amebocytes in primitive chelicerates, such as *Limulus polyphemus* and *Tachypleus* spp., except for the expression of proPO in Artemia hemocytes (Martin et al., 1999). Detailed studies of the cytoskeletons of *Artemia* hemocytes have also been reported, and these hemocytes have been implicated in phagocytosis in these animals (Day et al., 2000).

In decapods, GCs are the largest cell type, usually with a kidney-shaped nucleus and cytoplasm filled with membrane-bound, electron-dense granules. GCs have variable staining properties, and some cells are eosinophilic, whereas others stain less or faintly basophilic (Bauchau, 1981). Whether this differential staining reflects different developmental stages or some functional variability remains unknown, and the proportion varies with different environmental conditions.

Semigranular cells (SGCs) are smaller, typically possessing a larger central nucleus that is surrounded by cytoplasmic granules of