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Minireview

Mechanisms and roles of phagocytosis in *Drosophila* and *Caenorhabditis elegans*

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Abstract

Our understanding of the humoral immune response in both vertebrates and invertebrates has dramatically deepened in the past decade. In contrast, many of the mechanisms and roles of the cellular immune response remain to be elucidated. Phagocytosis is at the center of the cellular responses in both innate and adaptive immunity. Targets of phagocytosis are either invading microbes or altered self, that is, own cells that have become dispensable or harmful. The selective recognition and engulfment of target cells by phagocytes are achieved through the specific binding of receptors of phagocytes to ligands present on the surface of the target cells. However, these phagocytosis receptors and ligands are still being identified. The fundamental mechanism of phagocytosis appears to be the same in vertebrates and invertebrates, but whether or not genes are evolutionally conserved has yet to be determined.

Key words: apoptosis; *Caenorhabditis elegans*; *Drosophila*; innate immunity; microbial pathogen; phagocyte; phagocytosis

Introduction

Innate immunity is defined as a type of immune responses in which only products of genes already present in germ lines play roles, in contrast to adaptive immunity that uses both germ line genes and genes acquired by rearrangement of existing genes during development (Janeway and Medzhitov, 2002). Simpler organisms like invertebrate animals have only innate immunity, while organisms more complex than jawed fish possess both innate and adaptive immune systems. Innate immunity was once thought to be a prototype of the "more sophisticated" adaptive immunity, but it is now apparent that the two systems play individual roles and cooperate to protect against invaders and endogenous insults (Hoebe *et al*., 2004). Furthermore, the boundary between the two immune systems is becoming obscure (Flajnik and Pasquier, 2004). Given that either form of immunity consists of humoral and cellular responses, there are two types of humoral and cellular reactions in vertebrates and only one type in invertebrates. This raises the possibility that the roles of innate immunity in vertebrates and invertebrates are somewhat distinct. If so, a better way to fully understand innate immunity would be to compare the mechanisms and roles of immune responses between vertebrates and invertebrates.

Previous studies have revealed that at least the mechanistic part of the innate immune response is well conserved between vertebrates and invertebrates although the players are not exactly the same (Iwanaga, 2002; Brennan and Anderson, 2004). While the mechanisms and consequences of the humoral innate immune response have been intensively investigated particularly in insects and mammals (Janeway and Medzhitov, 2002), many issues still remain to be solved before we can gain a good understanding of the cellular response. Provided that the fundamental mechanisms of the cellular innate immune response are almost the same in vertebrates and invertebrates, a smarter way to address these issues is to begin with the analysis of genetically tractable invertebrate animals such as *Drosophila* and *Caenorhabditis elegans* (Mylonakis and Aballay, 2005). In this minireview, we summarize what is known of phagocytosis, an event at the center of cellular responses, in *Drosophila* and *C. elegans*, in an attempt to gain a deeper understanding of this phenomenon.

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Phagocytosis at a glance

Phagocytosis, a phenomenon whereby cells are engulfed and digested by other cells (Aderem and Underhill, 1999), is at the center of the cellular immune response in both innate and adaptive immunity; other cellular responses include cell-mediated killing in vertebrates, and cell-mediated killing and encapsulation in invertebrate animals. The cells in charge of phagocytosis are called phagocytes and consist of various types. Phagocytes are classified into two groups, professional and amateur cells. Professional phagocytes, macrophages as a representative, are full-time executors. In contrast, amateur phagocytes, which exert functions other than phagocytosis most of the time, exhibit phagocytic activity only when it is needed. There is another way to classify phagocytes; that is, phagocytes that circulate through the body and are responsible for phagocytosis in various places, and others that are localized to certain places and engaged in phagocytosis only there. The phagocytes of invertebrate animals have not been intensively characterized compared with those of vertebrates. In many invertebrates circulatory cells (either coelomocytes or hemocytes) exist, several types of which act as professional phagocytes. In the fruit fly *Drosophila*, plasmatocytes are such phagocytes and responsible for the phagocytic elimination of invaders and altered self in many areas of the body (Meister and Lagueux, 2003). Glia and some ectodermal cells also act as phagocytes in *Drosophila*. In contrast, there are no mobile phagocytes in *C. elegans*; the cells that simply neighbor target cells seem to carry out phagocytosis (Gravato-Nobre and Hodgkin, 2005).

Phagocytes selectively recognize and engulf cells that are foreign to our body or own cells that have become dispensable. The foreign cells are invading microbes, and the altered self includes cells that have become structurally and/or functionally spent, unwanted, aged, or harmful. Removal of the former targets is accomplished to eliminate microbial pathogens that may cause infectious diseases, while that of the latter is necessary for morphogenesis, establishment of tissue functions, and maintenance of tissue homeostasis, i.e. tissue renewal, avoidance of excessive cellular actions, and extermination of pathogenic or noxious materials (Stuart and Ezekowitz, 2005). Failure in the expeditious removal of such “unwanted” cells impairs normal development as well as increases the risk of infectious diseases, inflammation, or autoimmunity. Phagocytosis is induced when receptors present on the surface of phagocytes are activated by target cells (Aderem and Underhill, 1999) (Fig. 1). Upon the binding of target cells, the intracellular portion of phagocytosis receptors activates a signaling pathway, which in most cases leads to rearrangement of the actin cytoskeleton. As a result, the plasma membrane of phagocytes locally extends and surrounds the target, which is then ingested as membrane vesicles called phagosomes. There is another mode of phagocytosis whereby target cells appear to “sink” into phagocytes without any extension of the plasma membrane. It is presumed

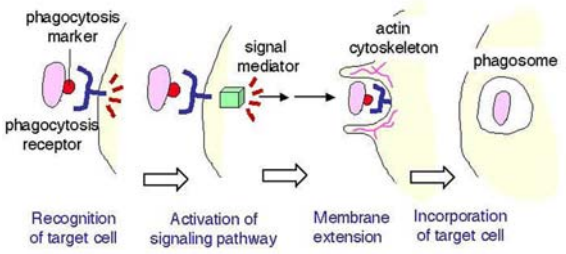

Fig. 1 *Recognition and engulfment of target cells by phagocytes.* Phagocytosis receptors of phagocytes bind to phagocytosis markers present at the surface of target cells. Marker-bound receptors activate a signaling pathway that leads to reorganization of the actin cytoskeleton. Portions of the plasma membrane of phagocytes then extend and surround targets. Finally, target cells are incorporated into phagocytes as phagosomes.

that the mode of engulfment varies depending on which receptors are responsible for the induction of phagocytosis as well as the size and shape of target cells (Champion and Mitragotri, 2006).

Target selectivity in phagocytosis is achieved through specific molecular recognition between phagocytosis receptors residing at the surface of phagocytes and their ligands or phagocytosis markers on the surface of target cells. The phagocytosis markers are either constituents of the surface of target cells or soluble molecules in body fluid that bind to the targets. The latter are called opsonins, being represented by immunoglobulin and complement of vertebrates that presumably do not exist in invertebrates: it is unclear if opsonin-dependent phagocytosis occurs in invertebrate animals (see below). Cell surface constituents specific for microbes are exemplified by a group of molecules called the pathogen-associated molecular patterns that are recognized by a group of receptors of immune cells, the pattern recognition receptor (Janeway, 2001). However, it is presumed that pattern recognition receptors and pathogen-associated molecular patterns, which are responsible for the induction of humoral innate immune responses, do not serve, at least in a direct way, as receptors and ligands, respectively, in phagocytosis. Mammalian receptors responsible for the phagocytosis of microbes have been characterized, including mannose receptors, Fc receptors, and complement receptors (Aderem and Underhill, 1999; Taylor *et al*., 2005). Mannose receptors directly recognize components of the bacterial cell wall, but Fc receptors and complement receptors bind to the opsonins immunoglobulin and complement, respectively. Several membrane proteins have recently been proposed to be receptors responsible for the phagocytosis of bacteria by phagocytes of *Drosophila*, but the corresponding bacterial ligands have yet to be identified (see below). Altered own cells are often induced to undergo apoptosis, a physiologic mode of cell death (Wyllie *et al*., 1980; Ellis *et al*., 1991), and become susceptible to phagocytosis (Savill *et al*., 1993; Savill and Fadok, 2000). Apoptotic cells

express phagocytosis ligands that do not exist at the surface of viable cells. These ligands are either endogenous molecules that move to the cell surface or pre-existing molecules at the surface whose structure or distribution changes during apoptosis (Lauber *et al*., 2004). The apoptosis-dependent structural reorganization of the cell surface has been well studied with mammalian cells, and the membrane phospholipid phosphatidylserine is the best-characterized ligand for phagocytosis receptors (Fadok *et al*., 1998; Schlegel and Williamson, 2001). Apoptosis-dependent expression of phosphatidylserine at the cell surface is also observed in cells of *Drosophila* and *C. elegans*. However, it is unclear whether phosphatidylserine serves as a phagocytosis ligand for apoptotic cells to be recognized by phagocytes of *Drosophila* and *C. elegans*. To date, no molecule has been identified as a marker for phagocytosis of altered self in *Drosophila* and *C. elegans*

Targets are incorporated into phagocytes as membrane vesicles called phagosomes, which are surrounded by the plasma membrane of phagocytes (Aderem and Underhill, 1999). The main fate of engulfed target cells is decomposition and digestion. Phagosomes are processed so that the engulfed targets are killed and degraded mainly by reactive species such as reactive oxygen species and reactive nitrogen species (Halliwell, 2006) and lysosomal enzymes, respectively (Fig. 2). A key step in the production of reactive oxygen species is the activation of an enzyme called NADPH oxidase (Underhill and Ozinsky, 2002; Geiszt and Leto, 2004). A prerequisite of the lysosomal degradation of engulfed target cells is the fusion of phagosomes with lysosomes that provide various enzymes for degrading components of engulfed cells. These processes are collectively called phagosome maturation and seem to be under a strict regulation.

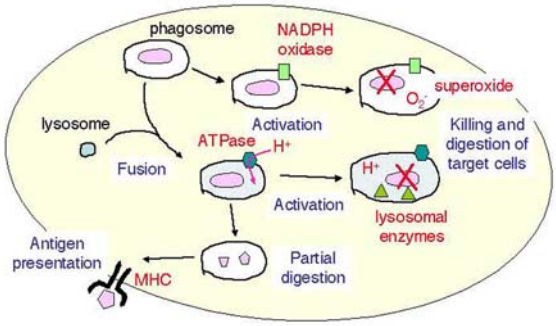

Fig. 2 *Fate of engulfed cells in phagocytes.* Engulfed cells are killed and digested by a reactive oxygen-mediated mechanism and lysosomal enzymes, respectively. Both reactions occur during structural and functional changes of phagosomes. Contents of engulfed cells are sometimes used as antigens for the activation of T lymphocytes and thus for the induction of adaptive immunity. MHC, major histocompatibility complex.

On the other hand, engulfed targets are sometimes not completely degraded but subjected to partial digestion. This occurs in a particular type of phagocyte, i.e. antigen-presenting cells such as dendritic cells in vertebrate animals, and processed components of engulfed cells are expressed at the surface together with the major histocompatibility complex for the activation of T lymphocytes (Ackerman and Cresswell, 2004). Microbes, some types of bacteria in particular, possess the ability to resist the actions of phagocytes at various steps (Ernst, 2000; Coombes *et al*., 2004). *Yersinia* inhibits phagocytosis itself through the actions of their own proteins that are delivered to phagocytes via the type III secretion system. *Salmonella* produce, after engulfment, proteins that inhibit the activation of NADPH oxidase in phagosomes. *Listeria* sneaks out of phagosomes by disrupting phagosome membranes using their own proteins. Other bacteria including *Leginonella* and *Chlamydia*, and the protozoa *Leishmania* also inhibit phagosome maturation. Phagocytes of the host organisms counterattack such microbes, in at least two ways as follows. Bacteria that have come out of phagosomes are surrounded again by membranes through a process called autophagy (Shintani and Klionsky, 2004), and phagocytes invaded by long-living microbes are often induced to undergo apoptosis and engulfed together with the microbes by other phagocytes. All these phenomena have been observed with mammalian phagocytes, and whether or not the same is true for phagocytes of invertebrate animals remains to be determined.

Phagocytosis of microbes in *Drosophila* and *C. elegans*

Phagocytosis of bacteria by Drosophila phagocytes

In *Drosophila*, most humoral immune responses are accomplished by cells of the fat body, a tissue equivalent to the mammalian liver, while blood cells called hemocytes are responsible for most cellular events (Hoffmann and Reichhart, 2002; Mylonakis and Aballay, 2005). There exist three cell lineages of *Drosophila* hemocytes, which emerge at different stages of development and participate in particular types of cellular immune responses (Meister and Lagueux, 2003). Two lineages, the plasmatocyte and the crystal cell, differentiate during the second half of embryogenesis. Plasmatocytes account for no less than 90 % of circulating hemocytes and act primarily as phagocytes. Therefore, these cells are considered to be responsible for the phagocytic elimination of invading microbes and altered self in *Drosophila*. Crystal cells are seemingly involved in humoral melanization mediated by phenoloxidase, but their role is not fully understood. The third hemocyte lineage emerges at the larval stage, and these cells called lamellocytes are responsible for the encapsulation of microbes, a cellular response often followed by phagocytosis.

Differently from mammalian phagocytes such as neutrophils and macrophages, how plasmatocytes act to phagocytose target cells has not been intensively studied. Even the identity of phagocytosis receptors and corresponding microbial ligands has been elusive. It is presumed that most phagocytosis

receptors of *Drosophila* phagocytes directly recognize molecules present at the surface of target microbes and apoptotic cells, because no molecules present in the hemolymph have been shown *in vivo* to serve as opsonin. Several proteins have been proposed to be phagocytosis receptors (Table 1) (Cherry and Silverman, 2006), although their ligands presumably present at the surface of microbes remain to be identified. The first receptor reported is known as a pattern recognition receptor. In *Drosophila*, a family of proteins called peptidoglycan recognition proteins (PGRPs) serves as pattern recognition receptors (Brennan and Anderson, 2004), as do Toll-like receptors in mammals (Akira *et al*., 2006). PGRP-LC, not PGRP-LA or -LD, has been identified as a protein, a decrease in the expression of which leads to a decrease in the level of phagocytosis of Gram-negative *Escherichia coli*, but not Gram-positive *Staphylococcus aureus*, by S2 cells (Rämet *et al*., 2002), a cell line established from hemocytes of *Drosophila* embryos. This approach, namely, a genome-wide screen with RNA interference-mediated inhibition of gene expression in phagocytes, was adopted by other investigators, and a group of proteins resembling a human protein called CD36 have been spotlighted. CD36 belongs to the class B scavenger receptor family (SR-B) that is responsible for the control of serum cholesterol levels as well as the removal of denatured serum proteins in mammals (Peiser and Gordon, 2001). Besides these actions, two SR-B proteins, SR-BI of mammals (Shiratsuchi *et al*., 1999) and Croquemort of *Drosophila* (Franc *et al*., 1996), have been shown to be involved in the phagocytosis of apoptotic cells. The genome-wide screen revealed that the *Drosophila* SR-B proteins Peste and Croquemort serve as receptors for the phagocytosis of bacteria by S2 cells. Peste targets *Mycobacteria* and *Lysteria* but not *E. coli* and *S. aureus* (Agaisse *et al*., 2005), but this specificity seems to change when Peste is expressed in mammalian cells (Philips *et al*., 2005). On the other hand, *S. aureus* is a preferred target for Croquemort in phagocytosis by S2 cells (Stuart *et al*., 2005). In addition, SR-CI, a class C scavenger receptor, of *Drosophila* seems to have some role in the phagocytosis of bacteria (Rämet *et al*., 2001; Philips *et al*., 2005). All the aforementioned proteins remain as candidate phagocytosis receptors at present, because their role *in vivo* in the phagocytic removal of bacteria is yet to be shown. Ezekowitz and colleagues extended their RNA interference screen with S2 cells, in which the transcription factor Serpent was found to be important for the phagocytosis of bacteria (Rämet *et al*., 2002). They searched for gene products whose expression is regulated by Serpent and examined their role in the phagocytosis of bacteria by S2 cells. Eventually one protein named Eater was found to bind to and engulf both *E. coli* and *S. aureus* (Kocks *et al*., 2005). Eater is a single-path membrane protein with epidermal growth factor (EGF)-like repeats in its extracellular portion, and is expressed primarily in plasmatocytes. Hemocytes prepared from mutant flies lacking the expression of *eater* showed a decreased level of the phagocytosis of both *E. coli* and *S. aureus*. Furthermore, the phagocytosis of those bacteria injected into the adult mutant flies was significantly impaired. The final candidate for a phagocytosis receptor of *Drosophila* is quite unique in that it possesses an immunoglobulin-like structure and is expressed as over one thousand isoforms through alternative splicing in hemocytes and the fat body (Watson *et al*., 2005). This family of proteins, named Dscam for immunoglobulin-superfamily receptor Down syndrome cell adhesion molecule, bind to *E. coli*, and larval hemocytes prepared from mutant flies with a reduced level of the expression of *dscam* showed less activity to phagocytose *E. coli* than

Table 1 Candidate receptors for the phagocytosis of bacteria by *Drosophila* phagocytes

Receptor name	Domains	*E. coli*	*S. aureus*	*Mycobacterium*	Other targets	*In vivo* evidence	References
PGRP-LC	peptidoglycan binding	yes	no	nd[1]	*M.luteus*[2]	nd[2]	Rämet *et al*., 2002
Peste	scavenger receptor[3]	no	no	yes	*Listeria*	nd	Philips *et al.*, 2005
Croquemort	scavenger receptor[3]	no	yes	nd		nd	Stuart *et al*., 2005
SR-CI	scavenger receptor[4]	yes[5]	nd	yes[5]		nd	Philips *et al*., 2005
Eater	EGF-like repeat	yes	yes	nd		yes[6]	Kocks *et al*., 2005
Dscam	Immunoglobulin-like	yes	nd	nd		yes[7]	Watson *et al*., 2005

1. Not determined;
2. Only viability of flies lacking PGRP-LC expression upon infection with bacteria was examined;
3. Class B scavenger receptor family;
4. Class C scavenger receptor family;
5. Extent of contribution is small;
6. Levels of phagocytosis of bacteria by larval hemocytes of flies lacking Eater expression are reduced. Levels of phagocytosis of bacteria injected into adult mutant flies are reduced;
7. Levels of phagocytosis of bacteria by larval hemocytes of flies lacking Dscam expression are reduced.

those from wild-type flies. In addition, its soluble form is present in the hemolymph. These findings evoke the possibility that Dscam serves not only as a receptor but also as an immunoglobulin-like opsonin in the phagocytosis of bacteria. More recently, a family of secreted proteins, called Teps for thioester-containing proteins, which serve as opsonins to mediate phagocytosis of microbes by S2 cells, was reported (Stroschein-Stevenson *et al.*, 2006). Of 6 Teps TepII, TepIII, and TepVI have been suggested to be involved in the phagocytosis of *E. coli*, *S. aureus*, and *Candida albicans*, respectively, though *in vivo* confirmation is required. Teps resemble the complement C3, and the presence of other complement-like proteins has also been noted though their action as opsonins remains to be shown (Lagueux *et al*., 2000; Kocks *et al*., 2003).

Phagocytosis of bacteria by C. elegans phagocytes

The nematode *C. elegans* maintained in laboratories propagates when fed with *E. coli*, and the lifespan of this worm is altered when the food source is changed to other bacteria. This suggests that *C. elegans* is immune to microbial pathogens. The study of innate immunity in *C. elegans* has begun with the analysis of the humoral immune response, and its mechanism and role have been shown to be basically the same as those in *Drosophila* and mammals (Gravato-Nobre and Hodgkin, 2005; Kim and Ausubel, 2005; Mylonakis and Aballay, 2005). Infection with some types of bacteria causes apoptosis in germ lines, and a mutant line of the worm lacking the expression of *ced*-3 and *ced*-4 is more sensitive to infection. This means that *C. elegans* uses the apoptotic pathway for innate immune responses against invading microbes. In contrast, the role of cellular responses in the protection of the worm from infectious diseases has not yet been settled. There exists a type of cell that serves as a phagocyte in *C. elegans*, but the importance of the phagocytic elimination of pathogenic microbes is expected to be small. The phagocytosis of invading microbes by *C. elegans* phagocytes is inefficient, simply because those phagocytes are not mobile. Further studies are needed to clarify the mechanisms and roles of the phagocytosis of microbes by *C. elegans* phagocytes.

Phagocytosis of apoptotic cells in *Drosophila* and *C. elegans*

Phagocytosis of apoptotic cells by C. elegans phagocytes

Although the contribution of phagocytosis to defense against the invasion of pathogenic microbes is unclear, apoptotic cells are definitely eliminated by phagocytosis in *C. elegans*. There are no circulating "professional" phagocytes in this worm, and cells that neighbor dying cells are in charge of phagocytosis. The pioneer work done by Horvitz and coworkers has revealed the existence of a set of genes responsible for the induction, execution, and regulation of programmed cell death or apoptosis in *C. elegans* (Ellis *et al*., 1991; Lettre and Hengartner, 2006). Such genes include those that play roles at the final stage of apoptosis, i.e. the engulfment and degradation of apoptotic cells (Gumienny and Hengartner, 2001;

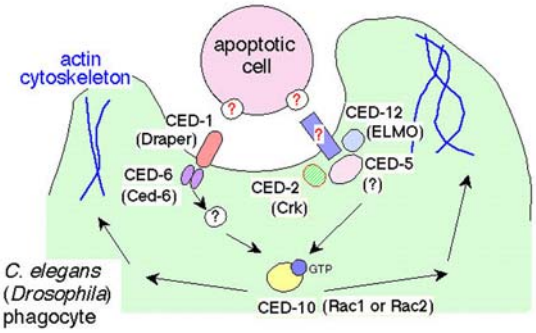

Fig. 3 *Signaling pathways for the induction of phagocytosis of apoptotic cells.* Two partly overlapping signaling pathways for the induction of phagocytosis of apoptotic cells, which were genetically identified in *C. elegans* and are considered to be conserved beyond species, are schematically presented. Shown in the parentheses are names of the *Drosophila* counterparts of the *C. elegans* proteins.

Reddien and Horvitz, 2004; Mangahas and Zhou, 2005). Seemingly there are two partly overlapping signaling pathways, which involve signal mediators conserved beyond species, for the induction of the phagocytosis of apoptotic cells by *C. elegans* phagocytes (Lettre and Hengartner, 2006) (Fig. 3), although a different opinion was recently provided (Yu *et al*., 2006). The onset of engulfment should be the activation of receptors residing at the surface of phagocytes. This occurs most likely by the binding of marker molecules of phagocytosis present on the surface of target apoptotic cells. Presumably there are two sets of phagocytosis receptor and ligand, but only one receptor has been identified to date. A single-path membrane protein named CED-1 has been genetically discovered and shown to serve as a phagocytosis receptor (Zhou *et al*., 2001).

There are counterparts of CED-1 in *Drosophila* and human, which are respectively called Draper (Freeman *et al*., 2003) and MEGF10 (Callebaut *et al*., 2003). Draper seems to be responsible for the phagocytic removal of apoptotic cells by *Drosophila* phagocytes (Freeman *et al*., 2003; Manaka *et al*., 2004) (see below), but whether or not MEGF10 plays roles in the clearance of apoptotic cells by mammalian phagocytes remains to be determined. CED-1 contains several structural domains, including EGF-like repeats, in the extracellular region and two segments containing tyrosine residues, which are candidate domains for protein-protein interaction, in the intracellular region. The former domain could serve as a site for the binding of an as-yet unidentified phagocytosis ligand, and the latter could be, most likely after phosphorylation of the tyrosine residues, a site for the assembly of downstream signal mediators such as CED-6 (Mangahas and Zhou, 2005). There has been no information regarding the identity of the other phagocytosis receptor. In contrast to the fact that many molecules have been proposed to be phagocytosis markers in mammals (Lauber *et al*., 2004), no molecules have

been shown to be ligands for phagocytosis receptors of *C. elegans*. *C. elegans* cells seem to express phosphatidylserine at their surface during apoptosis, but it is not known if externalized phosphatidylserine serves as a phagocytosis marker. The involvement of the *C. elegans* homologue of the mammalian phosphatidylserine receptor in the phagocytosis of apoptotic cells was reported, but the role for the mammalian protein itself as a phosphatidylserine-recognizing phagocytosis receptor is now doubted. It will be necessary to adopt an experimental strategy other than genetics for the identification of the other phagocytosis receptor and a couple of ligands, but the *C. elegans* system where mobile phagocytes and suitable cell lines are unavailable does not appear to be suitable for a rapid solution of these issues.

Phagocytosis of apoptotic cells by Drosophila phagocytes

In contrast to studies with *C. elegans* (see above) and mammals (Lauber *et al*., 2004), mechanisms of the phagocytosis of apoptotic cells in *Drosophila* have not been intensely investigated. Three membrane proteins have so far been proposed as receptors responsible for the phagocytosis of apoptotic cells by *Drosophila* phagocytes, but no molecules have been identified as phagocytosis markers presumably present on the surface of apoptotic cells.

Franc and coworkers were the first to identify a receptor responsible for the phagocytosis of apoptotic cells by *Drosophila* phagocytes (Franc *et al*., 1996, 1999). They searched for members of the C-type lectin family in larvae at the third instar stage and found a protein that belongs not to the C-type lectin family but to the SR-B family. This protein, named Croquemort, standing for “catcher of death”, is a membrane protein (single- or double-path) and expressed in hemocytes (plasmatocytes/lamellocytes) of embryos and larvae. Analyses of flies with a chromosomal deletion including the *croquemort* locus revealed that Croquemort is responsible at least in part for the phagocytosis of apoptotic cells, but not of bacteria, in *Drosophila* embryos, though the latter conclusion recently became controversial (Stuart *et al*., 2005). It is still unknown what molecule at the surface of apoptotic cells Croquemort binds to, and as to whether this receptor is contained in either one of the two signaling pathways (see Fig. 3) is not clear. Another molecule that has been shown to act as a receptor for the phagocytosis of apoptotic cells is Draper, the *Drosophila* homologue of the *C. elegans* phagocytosis receptor CED-1. Draper, a single-path membrane protein with EGF-like repeats, appears to serve as a receptor for the phagocytic elimination of apoptotic cells by both hemocytes and glia (Freeman *et al*., 2003; Manaka *et al*., 2004). Moreover, Draper is involved in the removal of axons by glia during metamorphosis for remodeling of the neural network and recovery from injury (Awasaki *et al*., 2006; Hoopfer *et al*., 2006; MacDonald *et al*., 2006). A ligand(s) for Draper remains to be identified for both apoptotic cells and degenerating axons, but phosphatidylserine is not likely to be the one (Manaka *et al*., 2004). The externalization of phosphatidylserine occurs also in *Drosophila* cells during apoptosis, but whether or not phosphatidylserine serves as a phagocytosis marker remains to be determined. The third candidate for a *Drosophila* phagocytosis receptor is a protein named Six microns under (Simu) that is expressed in hemocytes and glia (Kurant *et al*., 2006). The overall structure of Simu resembles that of Draper; both are single-path membrane proteins containing EGF-like repeats in the extracellular region. A decrease in the expression level of Simu in a hemocyte-derived cell line as well as in embryos leads to an increase in the number of apoptotic cells. Two structurally similar phagocytosis receptors, Draper and Simu, are co-expressed in hemocytes and glia, and how they cooperate with each other needs to be investigated.

Perspectives

In animals having both innate and adaptive immunity, cooperation between the two systems is necessary to maximize immune responses. For invertebrate animals lacking adaptive immunity, innate immune responses, either humoral or cellular, are more important than those in animals with both types of immunity. It is thus speculated that the role and mode of action of innate immune responses in invertebrate animals are somewhat different from those in vertebrate animals. Phagocytosis is at the center of cellular immune responses, and thus clarification of its mechanisms and consequences in invertebrate animals should lead to a better understanding of immunity in general.

There are many issues to be solved in order to achieve a full understanding of innate immunity in invertebrate animals. First, it is not known how these animals die upon being infected with some types of microbes. In mammals, septic shock is considered a consequence of excessive host responses to invading microbes, which are mostly mediated by proteins called cytokines secreted from immune cells. Is this also true for invertebrates? Probably the answer is yes, because cytokine-like proteins are secreted from *Drosophila* hemocytes when insults such as infections with microbes occur (Agaisse *et al*., 2003). The secreted proteins move via the hemolymph and stimulate the fat body, a *Drosophila* tissue equivalent to the mammalian liver, to produce stress proteins. There could thus be septic shock in invertebrate animals, at least in *Drosophila*. Taking into consideration that *Drosophila* produce immunoglobulin-like proteins (Watson *et al*., 2005) and complement-like proteins (Lagueux *et al*., 2000; Kocks *et al*., 2003; Stroschein-Stevenson *et al*., 2006), the architecture and operation of immunity do not seem to differ between invertebrate and vertebrate animals. It needs to be confirmed *in vivo* if these proteins act as opsonins to mediate the phagocytosis of microbes and altered self by *Drosophila* phagocytes. Conversely, it is necessary to determine to what extent the opsonin-independent phagocytosis of microbes and microbe-infected cells contributes to the immune response against pathogenic microbes in vertebrate animals. Another question to be answered is how invertebrates protect themselves against microbes other than bacteria, such as viruses and protozoa. This issue is also important for humans, because arthropods such as

insects can act as a vector for parasitic protozoa that cause severe infectious diseases. It is totally unclear how immune surveillance against invading Plasmodia, the parasite responsible for malaria, is accomplished in mosquitoes. A study on innate immune responses to viral infections has just started with *Drosophila* (Cherry and Silverman, 2006). The final general question is whether or not the contents of engulfed cells, microbes or altered self, are used to evoke further immune reactions in invertebrate animals. In mammals, cell contents are sometimes processed and presented as a complex with the major histocompatibility complex at the surface of specialized immune cells, antigen-presenting cells, for the activation of T lymphocytes (Ackerman and Cresswell, 2004). This seems unlikely to occur in invertebrates lacking adaptive immunity, but the presence of immunoglobulin-like molecules in *Drosophila* catches our imagination.

Leaving the above-mentioned questions for a future task, issues to be immediately addressed are: 1) to identify marker molecules that exist at the surface of bacteria and are bound by phagocytosis receptors of *Drosophila* phagocytes; 2) to identify a molecule that exists at the surface of apoptotic cells and is bound by *C. elegans* CED-1/*Drosophila* Draper; and 3) to identify the second phagocytosis receptor, after CED-1/Draper, and its ligand for the phagocytosis of apoptotic cells by phagocytes of *C. elegans* and *Drosophila*.

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