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Phagocytosis in Cellular Defense and Nutrition: A Food-Centered Approach to the Evolution of Macrophages

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Abstract

The uptake of macromolecules and larger energy-rich particles into the cell is known as phagocytosis. Phagocytosed material is enzymatically degraded in membrane bound vesicles of the endosome/lysosome system (intracellular digestion). Whereas most, if not all, cells of the animal body are equipped with the molecular apparatus for phagocytosis and intracellular digestion, a few cell types are specialized for a highly efficient mode of phagocytosis. These are the (“professional”) macrophages, motile cells that seek out and eliminate pathogenic invaders or damaged cells. Macrophages form the backbone of the innate immune system. Developmentally they derive from specialized compartments within the embryonic mesoderm and early vasculature as part of the process of hematopoiesis. Intensive research has revealed in detail molecular and cellular mechanisms of phagocytosis and intracellular digestion in macrophages. In contrast, little is known about a second type of cell that is “professionally” involved in phagocytosis, namely the “enteric phagocyte”. Next to secretory (zymogenic) cells, enteric phagocytes form one of the two major cell types of the intestine of most invertebrate animals. Unlike vertebrates, these invertebrates only partially digest food material in the intestinal lumen. The resulting food particles are absorbed by phagocytosis or pinocytosis and digested intracellularly. In this review we provide a brief overview of the enteric phagocytes described electron microscopically for diverse invertebrate clades, to then to compare these cells with the “canonical” phagocyte ultrastructure established for macrophages. In addition, we will review observations and speculations associated with the hypothesis that macrophages are evolutionarily derived from enteric phagocytes. This idea was already proposed in the late 19th century by Elias Metschnikoff who pioneered the research of phagocytosis for both macrophages and enteric phagocytes. We

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presume that modern approaches to better understand phagocytosis will be helped by considering the deep evolutionary relationship between the two cell types.

Keywords

Enteric phagocyte; Macrophage; Phagocytosis; Intracellular digestion; Evolution; Ultrastructure

1. Introduction

All cells devote a substantial fraction of their molecular components towards absorbing and assimilating (“digesting”) energy-rich material from their environment. For obvious reasons, the capability to absorb and digest is absolutely essential for cell survival and must have evolved in single celled organisms, which explains why many of the structural and regulatory molecules involved in these processes are highly conserved (homologous) among present day species.

Different pathways of absorption and digestion can be distinguished (Pollard et al. 2017). Large particles, such as cells and cell fragments, are “engulfed” by membrane protrusions (lamellipodia) in a process called phagocytosis. Smaller particles or macromolecules are taken into the cell through membrane invaginations, a mechanism called pinocytosis. Once inside the cell, phagocytosed or pinocytosed material ends up in a dynamic system of vacuoles where they are enzymatically degraded. This process, termed intracellular digestion, is complemented by extracellular digestion, where secreted enzymes degrade food particles in the extracellular space (e.g., lumen of the digestive tract). Small molecules resulting from extracellular digestion (e.g., amino acids, mono-/disaccharides) are taken up by the cell via diffusion or active transport.

Extracellular digestion is prevalent in multicellular animals, which have evolved complex digestive tracts with diverse cell types. For example, in the vertebrate digestive system, enzymes secreted by specialized glands (e.g., salivary gland, pancreas, intestinal crypts) carry out the first step of the digestive process (Fig. 1a). In a second step, a different set of enzymes associated with the apical membrane of the epithelial cells lining the digestive system (consistently referred to as “enterocytes” in this review) complete digestion, producing small molecules that are subsequently taken up through active transport (Hooton et al. 2015). Phagocytosis or pinocytosis (together called endocytosis), followed by intracellular digestion, plays a less prominent role in the vertebrate digestive system. This is different in most invertebrate taxa, where food is processed by a combination of extracellular and intracellular digestion (Fankboner 2001; Fig. 1b). Typically, the invertebrate digestive system is formed by a mixture of enzyme-secreting gland cells and enterocytes. The enzymes degrade food material to the level of cell fragments and macromolecules. Enterocytes then take up this pre-digested material by endocytosis, after which the digestive process is completed intracellularly. In other words, in many invertebrate phyla, enterocytes of the digestive system operate as effective phagocytes, which we will call “enteric phagocytes” in the following.

In addition to enteric phagocytes, a second class of phagocytic cells found in most animals, invertebrates and vertebrates alike, has become specialized for endocytosis and intracellular digestion: the macrophages of the innate immune system. Macrophages are mesodermally-derived, motile cells that absorb and digest foreign materials (e.g., macromolecules, bacteria, parasites) and cellular detritus that threaten the integrity of the body. As a matter of fact, much of the detailed information on endocytosis and intracellular digestion that is currently available stems from the study of macrophages. One would expect, based on their similar function, that macrophages (“immune phagocytes”) and enteric phagocytes share many common features in terms of ultrastructure and molecular pathways. However, this proposition can’t be confirmed or refuted because relatively little is known about the biology of enteric phagocytes. Part of the problem is that in vertebrates, dedicated enteric phagocytes associated with the digestive tract do not exist, or have not been defined as such. And among the invertebrates, the widely used “model systems” currently used for molecular-genetic studies, *Drosophila melanogaster* and *C. elegans*, have a gut in which enteric phagocytes are also not particularly prominent, even though intracellular digestion plays an important role (McGhee 2007; Miguel-Aliaga et al. 2018; see also Maduro, 2019, this issue). It is among the “up-and-coming” model systems, representing basal deuterostomes (ascidians, cephalochordates, hemichordates, echinoderms) and the diverse taxa of lophotrochozoa (e.g., annelids, molluscs, platyhelminths) and cnidaria, that we find enteric phagocytes as the dominant cell type of the digestive tract. To help preparing the way for studies in these animals, the current review attempts to provide a survey of the morphologies, documented electron microscopically, of the enteric phagocytes across the animal kingdom, and to compare these morphologies with the “canonical” phagocyte phenotype exhibited by macrophages. Morphologies are expressed in words, and the terminologies used by microscopists to describe the structural elements of the endocytotic and digestive “machinery” encountered in different enteric phagocytes are inconsistent. Presenting these varied terminologies in the attempt to discover an underlying phagocyte “gestalt” appeared to us as a worthwhile undertaking.

The second objective of the review is to address the possible phylogenetic relationship between enteric phagocytes and macrophages, an idea brought up in a recent hypothesis article (Broderick 2015). Given that nutritive phagocytosis exists in protists it can be surmised that during the evolution of metazoa, enteric phagocytes came first, and mesodermally derived macrophages second. Could the latter have evolved from the former? In the later sections of this review we will discuss findings of studies that address developmental and functional relationships between enteric phagocytes and motile, mesodermally derived macrophages (also called amoebocytes or coelomocytes in several instances) observed in many extant invertebrate species. We will start out by summarizing briefly the current concepts of intracellular digestion: how do the various organelles involved in this process relate to each other, and what is the terminology in place to describe them?

2. Morphological aspects of endocytosis and intracellular digestion: the endosome/lysosome system

The uptake of material into the cell, termed endocytosis, follows three different pathways, (micro)pinocytosis, phagocytosis, and macropinocytosis (Pollard et al. 2017; Fig.2a, b, b'). Fluids and particles measuring less than 0.5 μ m are taken up by pinocytosis; the ingestion of larger particles occurs by phagocytosis or macropinocytosis. In pinocytosis the membrane buckles inward in the shape of a small invagination ("pit"). Several different invagination mechanisms, notably clathrin-dependent endocytosis, caveolin-dependent endocytosis and clathrin/caveolin independent endocytosis, have been distinguished thus far (Mayor and Pagano 2007). Endocytotic pits pinch off the membrane to form the primary endocytotic vesicles, which measure 0.1-0.2 μ m in diameter (Fig.2a, b"). By contrast, in phagocytosis, microfilament-driven lamellipodia extend outward around the large particle ("cargo"; e.g., bacterium, cell, cell fragment) that is to be ingested (Rougerie et al. 2013). This process is orchestrated by the interactions between cargo surface molecules and receptors on the phagocyte which then recruit specific cytoskeletal effector molecules to particular sites of the plasma membrane. Macropinocytosis (Kerr and Teasdale 2009; Bloomfield and Kay 2016) describes a similar process, whereby cytoskeletal movements push out lamellipodia and membrane folds ("ruffles") which eventually "swallow" relatively big volumes of extracellular fluid, with or without particulate matter. As opposed to phagocytosis, macropinocytosis does not depend on specific ligand-receptor interactions. The vesicles resulting from phagocytosis and macropinocytosis are larger than the primary endocytotic vesicles that follow (micro)pinocytosis, and are termed phagosomes and macropinosomes, respectively (Fig.2a, b, c).

Once inside the cell, endocytosed material is channeled into the endosome/lysosome system. Endocytotic vesicles, phagosomes or macropinosomes dock at the early endosomes (EE), an elaborate network of interconnected vesicles and tubules located close to the plasma membrane (Huotari and Helenius 2011; Repnik et al. 2013; Pollard et al., 2017; Fig.2a). From here, certain parts of the internalized membrane is continuously recycled outward to the plasma membrane via recycling endosomes (Fig.2a). At the same time, vesicles carrying digestive enzymes and diverse membrane proteins arrive from the trans-Golgi network and merge with the EEs. As a result, large pieces of the EE vesicular network break off and are transported towards more central positions within the cytoplasm via microtubular transport (Bonifacino and Neefjes 2017). These so-called "carrier vesicles", aside from containing endocytosed food material and digestive enzymes, are characterized structurally by having many small internal vesicles enclosed within their lumen, and are therefore called multivesicular bodies (Gruenberg and Stenmark 2004; Pollard et al. 2017; MVBs in Fig.2a, b"). MVBs become part of another vesicular network, the late endosome (LE). Like EEs, LEs continuously exchange membrane-bound and intraluminal proteins with the trans-Golgi network. They differ from EEs by a number of specific membrane-bound proteins, such as the GTPase Rab7, which marks LEs, as opposed to Rab5, which is characteristic for EEs (Pollard et al. 2017; Fig.2a). In regard to differentiating between MVBs and LEs the literature is not yet consistent: ultrastructurally, both compartments are characterized by their "multivesicular" appearance. As a result, some authors use the terms LE and MVB

interchangeably (Huotari and Helenius 2011); others make the distinction between MVB (carrier vesicle) and LE (compartment targeted by, but separate from, carrier vesicle; Repnik et al. 2013).

The LE system interacts with yet another vesicular intracellular compartment, the lysosome. Lysosomes are round or irregularly shaped vesicles of 0.1-1 μ m diameter, filled with a homogenous, moderately electron-dense interior surrounded by a narrow, light halo (De Duve and Wattiaux 1966; Huotari and Helenius 2011; Lim and Zoncu 2016; Fig.2a, b'''). Lysosomes contain digestive enzymes, like acid phosphatase, and form part of all cells, but occur at greater density in cells that, like macrophages or hepatocytes, digest and store food materials. The fusion of lysosomes and LEs yields a hybrid vesicle, the endo-lysosome. This organelle, also called "secondary lysosome", is characterized by its heterogenous content that consists of electron-dense droplets of varying size and shape, as well as membranous vesicles and lamellar structures (Huotari and Helenius 2011; Pollard et al. 2017; Fig.2a, b'''). Like all other components of the endosome/lysosome system, endolysosomes also appear to be dynamic structures that undergo constant fusion-fission events. Parts of their membranes and enzymatic content get removed to reform lysosomes; vesicles derived from the trans-Golgi network carrying cargoes of "fresh" enzymes and membrane proteins fuse with the endolysosomes and lysosomes.

One should note that the structural appearance of EE, MVB, LE and endolysosome appears to be very different depending on the size of the initially endocytosed volume. Primary endocytic vesicles generated through pinocytotic membrane invaginations are similar in size to the vesicular structures of "typical" EEs, MVBs and LEs documented in the literature (Huotari and Helenius 2011; Fig.2b'', b'''). By contrast, phagosomes and macropinosomes (1-5 μ m) "dwarf" endocytic vesicles (Hard 1972; Buchmeier and Heffron 1991; Tjelle et al. 2000; Haas 2007; Kinchen and Ravichandran 2008; Fig.2a, c, c'). Once moving deeper into the cell, phagosomes remain large, rather than breaking down into smaller vesicles. Most ultrastructural descriptions of phagocytosis imply that phagosomes directly fuse with lysosomes into phagolysosomes (e.g., Kinchen and Ravichandran 2008). Intermediate carrier vesicles resulting from the processing of phagosomes, like MVBs (only much larger), have, to our knowledge, not been defined ultrastructurally. However, experimental studies clearly show that interactions between phagosomes and early/late endosomes via fusion and fission events do take place (Jahraus et al. 1998; Tjelle et al. 2000), suggesting that the intracellular digestion of endocytosed material, independent of size, follows similar pathways.

At the stage of the endolysosome/phagolysosome, food materials are broken down into small molecules that enter the cytoplasm to then exit the cell into the extracellular space, or to become part of anabolic biochemical pathways that synthesize macromolecules utilized in the cell, or deposited in specialized storage organelles. Prominent among these are the lipid droplets, or lipid bodies (LBs). Produced in and budded off from the endoplasmic reticulum, LBs are round, electron-dense vesicles surrounded by a specialized membrane that is formed by a lipid monolayer (Murphy 2001; Melo et al. 2011). In fixation protocols for electron microscopy lipids are often dissolved, leaving "empty spaces" of characteristic size, shape and distribution instead of the dense LBs (Fig.2d).

Not all of the food content of endolysosomes/phagolysosomes is broken down into monomeric small molecules. Many documented cases of intracellular digestion result in so called “residual bodies”, also called telolysosomes, which resemble phagolysosomes to a certain extent. Residual bodies also contain highly polymorphic granular and membraneous material (“myelin figures”), but are typically smaller, more irregularly shaped, and higher in electron density than phagolysosomes (Fedorko et al. 1968; Novikoff 1973; Essner and Haimes 1977; Del Conte 1979; Fig.2a, e). Residual bodies can accumulate in the cytoplasm as “lipofuscin granules”, or can be expelled from the cell by exocytosis (Kajihara et al. 1975; Hendriks and Eestermans 1986).

All of the structural components of the intracellular digestive pathway sketched out in the preceding paragraphs are intensively studied and known in considerable detail for vertebrate macrophages in the context of host defense. We will now turn to another cell type that employs the same pathway and, as will be shown further below, exhibits very similar ultrastructural features, but for a seemingly different purpose, namely nutrition. The cell type in question is the enteric phagocyte, which makes up a major fraction of intestinal cells found in most invertebrate phyla. Interestingly, at the time when phagocytosis was first discovered, both mesodermally derived macrophages and endodermal enteric phagocytes were very much considered to be part of the same functional system, and to be closely related ontogenetically and phylogenetically, a theme we will briefly explain in its historical context next, and then later return to towards the end of the review where we address functional and developmental relationships between the two cell types.

3. Enteric phagocytes: a historical introduction

In 1878, Elias Metschnikoff described the intestinal cells (also called gastrodermal cells) of various platyhelminth (flatworm) species as “cylindrical amoeboid cells” capable of ingesting food particles, such as blood cells and liver cells (Metschnikoff 1878). He also surveyed many other invertebrate organisms (Metschnikoff 1884), looking at both intestinal (endodermally derived) cells, as well as stromal (mesodermally derived) cells involved with the uptake and digestion of particles, a process for which he coined the term “phagocytosis” (Fig. 3a, b). Historically, Metschnikoff’s scientific work has to be understood in context of the coming-together of embryology and the (then new) concept of Darwinian evolutionary theory (Tauber 2003). Events shaping the embryonic development of an organism were seen as directly linked to the phylogenetic history of that organism. Furthermore, it was understood that evolution was driven by natural selection, the “survival of the fittest”. Embryologists started to apply this idea for the cells of a (developing) organism, which, they speculated, compete with one another for space and resources. The diverse cell types that originated in a multicellular animal were seen as part of an overall “disharmonious” organism, where the phagocytosis of one cell (of the same organism, or taken in as prey) by another cell was a major cellular activity, directed at regulating proper cell numbers, and at the same time providing energy. Metschnikoff paid much attention to the massive cellular break down that occurs during metamorphosis of many animals, where motile macrophages moving throughout the body cavities and stroma ingest the cellular debris. He also emphasized his findings that in many invertebrates cells of all germ layers, including ectoderm and mesoderm, carry out phagocytosis (Metschnikoff 1884). Macrophages and

intestinal phagocytes were seen as being closely related, with stromal macrophages moving in and out of the intestinal epithelium, or (stationary) intestinal cells passing on ingested food particles to (motile) stromal cells, which then further digest and distribute these particles all over the body. Metschnikoff even coined the word “phagocytoblast” for the combined endodermal and mesodermal germ layer.

Descriptive and experimental studies of many researchers in the early decades of the 20th century focused on intracellular digestion carried out by enteric phagocytes (reviewed in Yonge, 1937). However, before the use of electron microscopy, cellular events involved in phagocytosis were understood only at a very rudimentary level. It was assumed that particles were taken up into the cytoplasm, and were only later (in some cases) incorporated into a fluid-filled vacuole, termed food vacuole, or digestive vacuole (e.g., Hirsch 1925; Millot 1938). Aside from this compartment (nowadays termed phagosome or phagolysosome), all other organelles were too small to be observed light microscopically. On the other hand, secretory granules of secretory (gland) cells could be resolved in the light microscope, and histo-chemical techniques were developed to characterize different types of secretions. It became clear that in most animals, the intestinal epithelium is comprised of a mixture of gland cells and absorptive cells acting as phagocytes; the digestive process was characterized as a hybrid of extracellular and intracellular digestion (Yonge 1937; see Fig.3c). Gland cells secrete enzymes that partially break down the food material; the products are intracellularly digested by enteric phagocytes. It remained an open question (and still is an open question to the present day, for most animals) whether the uptake of small molecules through specialized channels and transporters is a function of the same enteric phagocytes, or whether other cell types dedicated to this function exist.

The arrival of electron microscopy in the 1950ies made it possible to resolve the architecture of cytoplasmic organelles, including the interconnected tubules and vesicles that make up the “vacuome” (endoplasmic reticulum, Golgi apparatus, endosomes, lysosomes) of the cell. Lysosomes as carriers of intracellular digestive enzymes were isolated from hepatocytes for the first time in 1955 (De Duve et al. 1955) and soon shown to be part of other cell types in all organisms (De Duve and Wattiaux 1966). Electron microscopic investigations of the intestinal tract of many animal taxa confirmed the earlier established notion of a combined extracellular/intracellular digestive system carried out by an admixture of gland cells and enteric phagocytes, and documented the diversity of membrane-bound organelles associated with intracellular digestion (Fig.3d, e). Several researchers, aware of the ongoing biochemical-ultrastructural work addressing the dynamic system of membrane-bound intracellular compartments in vertebrate cells, employed the “modern” nomenclature [e.g., endocytic vesicles/phagosomes, primary lysosomes, secondary lysosome (=phagolysosome)] to describe what they observed in invertebrate enteric phagocytes. For example, Riley (1973) investigating nematodes, and Owen (1972) in molluscs, explicitly referred in their work to the system of terms and functional relationships introduced by cell biologists that studied vertebrate cells at a molecular level (Fig.3d, e). However, overall, the literature describing the ultrastructure of enteric phagocytes employs a nomenclature that is somewhat varied and often vague. Given the fact that experimental studies addressing the function and interrelationships of the different organelles observed are difficult or impossible to perform, this lack in terminological clarity and/or precision is of course understandable. Table 1

provides an overview of the terminology used to describe the organelles of intracellular digestion in electron microscopic investigations of the digestive system of different invertebrate clades.

4. Structure and distribution of enteric phagocytes

Enteric phagocytes are polarized epithelial cells joined together by an apical junctional complex that, in most cases, is described as a combined zonula adherens and septate junction. The apical membrane facing the gut lumen bears conspicuous cilia and/or microvilli, as summarized for the different invertebrate clades in Fig.4 (for references, see Table 1). If microvilli are present, they often form a dense array of evenly spaced elements that, based on its light microscopic appearance, is called brush border. Non-bilaterian clades (ctenophores, cnidarians) typically have ciliated (or flagellated) gastrodermal cells with more sparse microvilli. The same is the case for the choanocytes and ventral epithelial cells of sponges and placozoans, respectively; these cells may functionally be considered to represent enteric phagocytes, without necessarily implying homology. For the enteric phagocytes of deuterostomes, with the exception of ascidians and vertebrates, a dense brush border is accompanied by cilia. The same applies to most lophotrochozoan phyla. In some phyla (e.g., nemertines), microvilli appear to be missing, whereas in others, (e.g., gastrotrichs, bryozoans) cilia may be absent. Ecdysozoa also appear to lack cilia in their gastrodermal cells.

Enteric phagocytes and secretory gland cells (as well as some other cell types, including endocrine cells, stem cells, and neurons, which will not be further considered in the following) typically occur intermingled in the middle segment (midgut) of the digestive tract (Fankboner 2001). In most bilaterian animals, the digestive tract is divided into an anterior foregut, middle midgut and posterior hindgut (Brusca et al. 2016). The foregut (pharynx, esophagus) is often specialized for functions of food comminution (e.g., milling, crushing, shredding) and food storage; it also has sometimes glands secreting digestive enzymes or mucus. The midgut is the location of food digestion and absorption, and the hindgut subserves the resorption of water and excretion of wastes. The digestive system of all animals, excepting the sponges and placozoans, forms an elongated cylinder (“gut” or “intestine”) with a single opening (cnidarians, xenacoelomorphs, platyhelminths, gnathostomulids) or a separate mouth and anal opening (all other phyla). In many clades, blind-ending sacs (“diverticula” or “caeca”) branch off the midgut (Brusca et al. 2016). These usually represent compartments that are particularly rich in phagocytes dedicated to food absorption and digestion (intestinal caeca in echinoderms, bryozoans, chelicerates and hexapods; intestinal diverticula in nemertines; pyloric gland in ascidians; hepatic sacculations or hepatic caecum in hemichordates and cephalochordates, respectively; hepatopancreas in molluscs and crustaceans). In some cnidarians, notably the Anthozoa, the different cell types involved in digestion (glandular cells, phagocytes, purely resorptive cells) are spatially separated in longitudinal “tracts” that extend along the edges of the mesenteries and mesenterial filaments (Van-Praët 1985; see also Steinmetz, 2019, this issue).

For some animal groups a single cell type which appears to carry out the function of both (enzyme) secretion and absorption/digestion dominates the intestinal epithelium. This has

been observed for a number of ecdysozoan phyla, notably priapulids (Storch et al. 1989; Storch 1991), nematodes (Lee 1969; Riley 1973; Wright 1991), and most hexapods (see Holthof et al. 2019 and Caccia et al 2019 this issue). In priapulids and most nematodes, hallmarks of intracellular digestion (e.g., phagosomes) were seen in these cells. In the same cells, secretory vesicles originating within the ER and fusing with the apical membrane to release their content were observed (Riley 1973). The characteristic electron dense secretory granules characteristic for gland cells in general are rare or absent from intestinal cells of nematodes and insects. It has been speculated that the process of enzyme concentration, which results in the dense secretory granules, is shortened or omitted in these animals (De Priester 1971; Richards and Richards 1977; Bignell et al. 1982) and secretions are discharged continuously.

A characteristic shared by macrophages and enteric phagocytes in many animals is their tendency to fuse into multinucleate syncytia (or, terminologically more correct, plasmodia). This was already emphasized by Metschnikoff (1884), and confirmed in several consecutive studies, notably in members of the clades Platyhelminthes (Holt and Mettrick 1975), Phoronida (Herrmann, 1997) and Cnidaria (for medusa of *Clytia hemispherica*: V.H., unpublished observation). It is possible that large multinucleate cells are more effective in phagocytosing large food particles. The tendency towards forming a digestive syncytium is taken to an extreme in most members of the Acoela (members of the Xenacoelomorpha; see Gavilan et al., 2019, this issue). Here, the entire “central parenchyma” consists of a mass of merged phagocytic cells, and is able to “swallow” entire prey animals, such as nematodes or copepods, into gigantic phagosomes. We would argue that researching the ultrastructural and biochemical details of intracellular digestion in acoels will reveal many novel and interesting aspects of this process.

5. The diversity of organelles of intracellular digestion in enteric phagocytes

The uptake of food particles by canonical phagocytosis, as defined by the extension of lamelliopodia around the particles, followed by their engulfment and sequestrations into large subapical phagosomes, has been described for sponges, ctenophores, cnidarians, acoels, platyhelminths, nemertines, and cephalochordates (Fig.4, type “B”; Fig.5a-c; see also Tab.1 and references therein). In planarians (platyhelminths) fed with liver, entire liver cells are ingested. After only minutes post-feeding, phagosomes, often called “food vacuoles” based on the older term introduced in the light microscopic literature, appear in the subapical cytoplasm. They measure in excess of 5µm and consist of a double membrane tightly enclosing the engulfed cell (Garcia-Corrales and Gamo 1988; Fig.5a). Approximately one hour post-feeding, phagosomes move deeper into the cell where they fuse with multiple, small lysosomes to become phagolysosomes, also termed “digestive vacuoles” or “secondary lysosomes” by various authors (Tab.1 and references therein). Phagolysosomes are similar in size to phagosomes, but differ in the appearance of their content. Thus, the integrity of the ingested cell has broken down, giving rise to irregular clumps of electron-dense granular and membraneous material. Several hours later, the content of phagolysosomes further condenses to morph into a more or less heterogeneous matrix of

electron-dense material. The resulting organelle constitutes the residual body. In planarians and some other species, residual bodies were observed to move apically and then being released into the gut lumen exocytotically, similar to what has been observed for sponges and unicellular organisms (Willenz and Van de Vyver 1982; Garcia-Corrales and Gamo 1988; Fig.5c). This exocytotic “cellular defecation” process occurring in enteric phagocytes is also known for vertebrate cells (e.g., hepatocytes: Kerr 1970; Munnell and Cork 1980; macrophages: Song and Hanayama 2016).

Nutritive phagocytosis often follows a mechanism (called “B” in Fig.4; Fig.5d-g) that deviates, at least in its beginning stages, from the canonical pathway described above. Thus, food particles that are smaller in size than whole cells, are taken up through membrane invaginations into small subapical endocytic vesicles to which a plethora of different terms have been applied (e.g., microvesicle, pinocytotic vesicle, coated vesicle, discoideal flattened vesicle, pinosome, among others; see Table 1 and literature therein). As a next step, these endocytic vesicles fuse with each other into larger vacuoles, variably termed macrovesicles, food vacuoles, phagosomes, large endosomes, among others (Owen 1970; Owen 1972; Riley 1973; Filimonova 2008). The merging of lysosomes to these phagosomes results in phagolysosomes (“digestive vacuoles”), which move deeper into the cell and are processed further as described above. Experimental studies, using application of small particles like graphite, thorotrast, or nanoparticles (see Table 1), confirmed the initial uptake of these materials via membrane invaginations into small vesicles, which then transferred them to large endosomes. This type of “pino-phagocytosis” is less frequently discussed in studies of macrophages or other phagocytotic cell types in vertebrates, where a “microvesicular pathway” (pinocytosis > pinosome > endosome > endolysosome) appears to exist separately from a “macrovesicular” pathway (phagocytosis > phagosome > phagolysosome). However, the merging of pinocytotic vesicles into larger phagosomes is known (e.g., Straus 1964, for kidney cells), and has been documented in detail for a peculiar absorptive cell type that exists in the intestine of newborn mammals (Wilson et al. 1991; Fig. 5h). These absorptive cells ingest protein complexes by pinocytotic vesicles that become part of the early endosome. Maturing to late endosomes (“multivesicular bodies”), the vesicles fuse with each other, and with lysosomes, into a “giant lysosome” (phagolysosome) located in the supranuclear cytoplasm. It is tempting to speculate that this endocytotically active mammalian enterocyte, which only exists in the perinatal period, is an evolutionary vestige of the enteric phagocyte, which dominates the digestive system of invertebrates, including that of ascidians and cephalochordates, the closest vertebrate relatives.

Residual bodies have been described for enteric phagocytes of both the “canonical” type (A) and the pino-phagocytotic type (B; see Table 1 and references therein). Residual bodies can adopt different shapes and, accordingly, received different names, such as (among others) brown body vacuole, pigment granule, and spherical inclusion. Aside from residual bodies, energy storage granules, notably lipid bodies and glycogen granules, exist in most enteric phagocytes documented in the literature. Glycogen granules are arrays of densely packed, electron-dense “grains” of 15-40nm diameter (Revel et al. 1960). Lipid bodies are larger spherical inclusions, occurring singly or merged into tightly packed “grapes”. With lipids still in place they are electron-dense; more often, lipids are dissolved by the embedding process, and the lipid bodies appear as clear spherical spaces. Lipid bodies were sometimes

interpreted as directly emerging out of the phago-lysosomal digestive process. For example, in the detailed analysis of enteric phagocytes in the cnidarian *Tripedalia*, Chapman (1978) concludes that “ingested material is progressively transformed into free basal droplets”. The author’s interpretation is based on the occurrence of lipid inclusions, within phagolysosomes, of similar size and texture as the free lipid bodies. However, given our current knowledge of lipid synthesis and the Golgi/Endoplasmic Reticulum/Lysosome (GERL) system in general, this interpretation is unlikely to be correct; rather, lipid bodies arise from the ER where fatty acids released from phagolysosomes are used to synthesize triglycerides (Murphy 2001; Melo et al. 2011).

6. Interrelationship between enteric phagocytes and macrophages

We discussed in the above historical introduction how at a very early stage in the research on phagocytosis, Metschnikoff (1884) and other observers emphasized the close functional and ontogenetic/phylogenetic relationship between enteric phagocytes and macrophages. In sponges, considered to be among the most basal metazoan clades, macrophages, called archaeocytes, are directly involved in the uptake of algae and other single-celled food organisms. In several other invertebrate phyla, motile macrophages in the stroma or coelom take up cellular debris resulting from dying cells (e.g., during metamorphosis) as well as particles injected into the body cavity. Aside from this direct participation in food uptake, macrophages, which actively move throughout the body, were thought to play an important role in distributing digested food stuffs, receiving this material from stationary phagocytes lining the gut, and transporting it to other tissues.

Electron-microscopic studies have largely confirmed these early observations and speculations. In sponges, stationary choanocytes and pinacocytes and motile archaeocytes all interact during the process of food uptake and intracellular digestion (Weissenfels 1976; Imsiecke 1993; Fig.6, left). Bacteria or single-celled algae enter the sponge body through ingestive channels, and are taken up into large phagosomes within pinacocytes (the cells lining ingestive channels), choanocytes, and archaeocytes. Archaeocytes move in between choanocyte chambers and canals and exchange phagosomes (or phagolysosomes) with choanocytes/pinacocytes, whereby plasma membranes of two cells come into close contact, the “donor cell” exocytoses the phagosome, and the “recipient cell” endocytoses the content of the phagosome. In the same manner, residual bodies (“digestive remnants”; Imsiecke 1993) are exchanged between cells, and eventually exocytosed into the excurrent canals. Aside from their involvement in absorption and digestion of nutrients, archaeocytes also give rise to cell types forming spicules, and providing immunity (Funayama et al. 2005).

Stromal cells with attributes of phagocytes, containing lysosomes, phagolysosomes and nutrient storage organelles, have also been observed in several bilaterian clades, notably the platyhelminths (flatworms). In these animals, the stroma (paradoxically called parenchyma in the classical literature) is formed by large cells with abundant processes that interdigitate with and surround other cells (e.g., neurons, muscles, glands). Like gastrodermal, enteric phagocytes, these stromal cells also show phagocytotic activity when challenged with experimentally applied particles, and are thought to be involved in the uptake and distribution of intracellularly digested food material or symbiotic microalgae (Haffner 1925;

Jennings 1957; Pedersen 1961; Morita and Best 1984; Rieger 1991; Fig.6, center). In acoels, formerly counted as members of the Platyhelminthes but now considered part of a separate superphylum, Xenacoelomorpha (see Gavilan et al. 2019, this issue) the role of absorbing gastrodermal phagocytes and distributing stromal macrophages has been combined in a single multinucleated cell type, the digestive syncytium. Here, endodermal cells merge into a syncytial mass that takes up whole prey animals into large phagosomes. At the same time, the digestive syncytium forms abundant lamellar processes that reach virtually every cell of the body, and therefore is in a position to distribute the nutrients widely among all tissues.

In most bilaterian animals the mesoderm forms epithelial layers surrounding inner cavities, called coelomata (singular: coelom). Spaces in between coelomata become the vascular system (Ruppert and Carle 1983; Hartenstein and Mandal 2006). Specialized motile, mesodermally derived cells populate the coelomata (“coelomocytes”) and the vessels (“hemocytes” or blood cells). Cells with the characteristics of macrophages are prominent among both hemocytes and coelomocytes (Hartenstein 2006). Macrophages move throughout the body cavity and are able to invade the stroma of all organs. Invertebrate macrophages have been studied extensively in the context of development and innate immunity (Cooper 2010; Vlisidou and Wood 2015; Abnave et al. 2017). During development, they represent the “professional macrophages” that eliminate apoptotic cells. In addition, they cooperate with humoral factors to battle invading parasites and microbes. Since many of these invaders enter through the digestive tract they typically first encounter gastrodermal cells. The complex interactions between macrophages and gastrodermal cells that occurs as part of the immune response has been studied in some detail in echinoderms. In sea urchin larvae, pathogenic bacteria introduced into the gut cavity evoke the release of cytokines from gastrodermal cells, which in turn recruit certain types of coelomocytes (pigment cells) towards the gut epithelium (Buckley and Rast 2017; 2019, this issue; Fig.6, right). According to a number of studies (e.g., Chia and Koss 1991), coelomocytes appear to become integrated into the gut epithelium. Their possible participation in nutritive phagocytosis, aside from immune-related processes, seems likely and has been reported in the classical literature (van der Heyde 1922; Kindred 1924), but has not been experimentally assessed in the recent literature. Phagocytic amoebocytes shuttling between the coelomic cavity and the intestine (where they become transiently integrated into the epithelium) have also been observed in several annelid species (Kermack 1955; Marsden 1963, 1966).

7. Ontogeny and phylogeny of phagocytes

Coming towards the end of our review we would like to ask whether the close structural and functional relationships between enteric phagocytes and macrophages are also reflected in the ontogeny and phylogeny of these cell types. In animals that branched off close to the base of the metazoa, the two types of phagocytes may simply represent different developmental stages of the same cells. This can still be observed in extant porifera, in which the archaeocytes of the mesohyl (the mass of extracellular matrix and cellular elements filling the space in between pinacoderm and choanoderm) can convert into choanocytes (enteric phagocytes), and vice versa (Nakanishi et al. 2014; Sogabe et al. 2016; Fig.7, bottom center). Note that, while it appears clear that archaeocytes are involved in immunity (Funayama et al. 2005), molecular evidence for the homology between these cells

and bilaterian macrophages has not yet been provided. In cnidarians, such as the soft corals (octocorallia), phagocytic amoebocytes with important immune functions populate the mesoglea, the extracellular matrix in between epidermis and gastrodermis (Menzel et al. 2015; Fig.7, bottom right). It is unknown whether these cells are derivatives of the gastrodermis, or could even develop directly from enteric phagocytes.

In bilaterian animals where macrophage development has been studied, these cells form part of mesodermally derived lineages. Their development is embedded in the process of hematopoiesis, which starts out in pockets of cells that are typically associated with the mesoderm flanking the gut [visceral mesoderm, or splanchnopleura; Ruppert and Carle 1983; Hartenstein and Mandal 2006; Fig.7, top right). In the most simple scenario found, for example, in polychaete annelids, specialized domains within the splanchnopleura (and to a lesser extent, the somatopleura) exhibit higher rates of proliferation and extrude coelomocytes and blood cells into the lumen of the coelom or blood vessels, respectively (Eckelbarger 1976; Dales and Dixon 1981). More typically, one observes a hematopoietic mechanism whereby stem cells delaminate from the splanchnopleural epithelium and aggregate to form compact hematopoietic organs, called lymphoid organs or lymph glands. These structures are attached to the coelomic wall or large blood vessels, as seen, for example, in representatives of annelids, molluscs, and arthropods (Hoffmann et al. 1979; Hartenstein 2006; Grigorian and Hartenstein 2013). In vertebrates, macrophages (among other blood cell types) are initially formed from the embryonic endothelia (“hemogenic endothelia”) associated with the yolk sac and the large blood vessels (e.g., aorta; McGrath et al. 2015). In addition, the hemogenic endothelia produce hematopoietic stem cells (HSCs) that settle in the liver and bone marrow and form a permanent, self renewing source of monocytes and other blood cells. Another group of immune-related macrophages are the tissue resident macrophages found in the brain (microglia) and other organs (e.g., Kupffer cells in the liver). These cells derive from early embryonic hematopoietic cells (other than HSCs) that settle in the organ primordia where they replicate in situ.

Despite of the fact that, at the time when they are specified as distinct cell types, enteric phagocytes and macrophages of bilaterians reside in different tissue layers (i.e., endoderm-derived digestive epithelium and mesoderm-derived stroma), one may still recognize genetic and phylogenetic affiliations between these cells. Phylogenetically, mesoderm is thought to have evolved from within the endoderm (Rodaway and Patient 2001; Grapin-Botton and Constam 2007). In cnidarians, such as the sea anemone *Nematostella vectensis*, the endodermal layer that gives rise to the gastrodermis expresses many genes that, in bilaterian animals, occur in mesodermal derivatives. Specifically, the so-called mesenteries of *Nematostella*, which contain cells functioning in reproduction (somatic gonadal cells) and locomotion (parietal and circular muscle fibers), express many mesodermal determinants, such as *mef2*, *six4/5*, *tbx1/10*, *foxC*, *nkx3/bagpipe*, and others (Steinmetz et al. 2017; Steinmetz, this issue). It is possible that during the early evolution of the bilateria, certain sets of cells with functions like locomotion, reproduction and immunity separated from the endoderm to be better able to perform their functions as contractile fibers or motile macrophages. Eventually this separation was brought forward in development, resulting in the establishment of a distinct middle germ layer, the mesoderm, in the early embryo. From that time onward, enteric macrophages and macrophages formed as ontogenetically separate

cell lineages (Fig.7, top right). Note also a more far-reaching version of this hypothesis (Steinmetz 2019, this issue) according to which the entire endoderm, including the enteric phagocytes, of the cnidarian/bilaterian ancestor became the mesoderm, and the (ectodermal) pharyngeal epithelium gave rise to the bilaterian endoderm. In this scenario, enteric phagocytes found in the bilaterian gastrodermis would have to evolve convergently from other cell types of the gastrodermis, and/or descend from mesodermal macrophages resettling the gut.

Assessing the homology of cell types across different clades relied, traditionally, on the comparison of their ultrastructure (i.e. from TEM). The introduction of sequencing methodologies applied to single cells offers a new possibility for classifying cells using their transcriptional profiles (Sebé-Pedros et al. 2018). The assumption is that similar profiles should be good indicators of their “homology” (Moroz 2018; Arendt et al 2019). Does this approach add to clarify the relationship between enteric phagocytes (such as those existing in planarians), and vertebrate macrophages? In the two systems where extensive transcriptomic analysis has been done to characterize the diversity of macrophage/phagocyte cell types, the flatworm *Schmidtea mediterranea* (Plass et al. 2018 and Fincher et al. 2018)) and the mice lung tissue (Aran et al. 2019) the datasets are mostly identifying highly expressed, structural or metabolic, components (and thus, mostly inconclusive for homology assessments); a fact that highlights the difficulties in comparing cell types across widely divergent clades. In fact, it is becoming clear that homologous cell types often don't show highest transcriptome similarity (even when comparing cell types of known homology between different vertebrates; see: Liang et al, 2018). The fact that transcription factors are absent or rarely present from these datasets (a consequence of the low levels of expression) points to the urgent need of using alternative methodologies directly targeted to the identification of transcriptional regulators and their functional interactions (Gene Regulatory Networks; GRNs) which should provide more solid proofs of homology. Needless to say, a framework of embryological origin should be taken into account, since they can inform us of putative convergence effects. The use of concepts such as “character identity networks” (Wagner, 2007), “synexpression groups” (Gawantka et al., 1998) or “network kernels” have been suggested as better representatives of the cells or tissue “deep homologies” (Davidson and Erwin, 2006). One relevant example here is the modeling effort carried out by Collombet and collaborators (Collombet et al. 2017) aiming at understanding the specification of different hematopoietic lineages, in particular the differentiation of macrophages (monocytes) and B-lymphocytes from a common MB progenitor. These authors identified 23 factors, most of them transcription factors, but also a few cytokine receptors, that were preferentially expressed in the progenitors, macrophages, or B cells. Experimentally tested expression patterns and genetic interactions place the factors into a complex web that represents a “macrophage identity network”. The large majority of the factors is conserved in *Drosophila*, and, in part has retained a role in the specification of fly macrophages. Most factors are also found in other bilaterians; for example, about half of the factors were identified in the transcriptome of the acoel *Symsagittifera roscoffensis* (Philippe et al 2019). Expression data and functional studies will help to discern whether the “macrophage identify network” also plays a role in the specification of enteric phagocytes, which would further confirm a common evolutionary origin of the two cell types.

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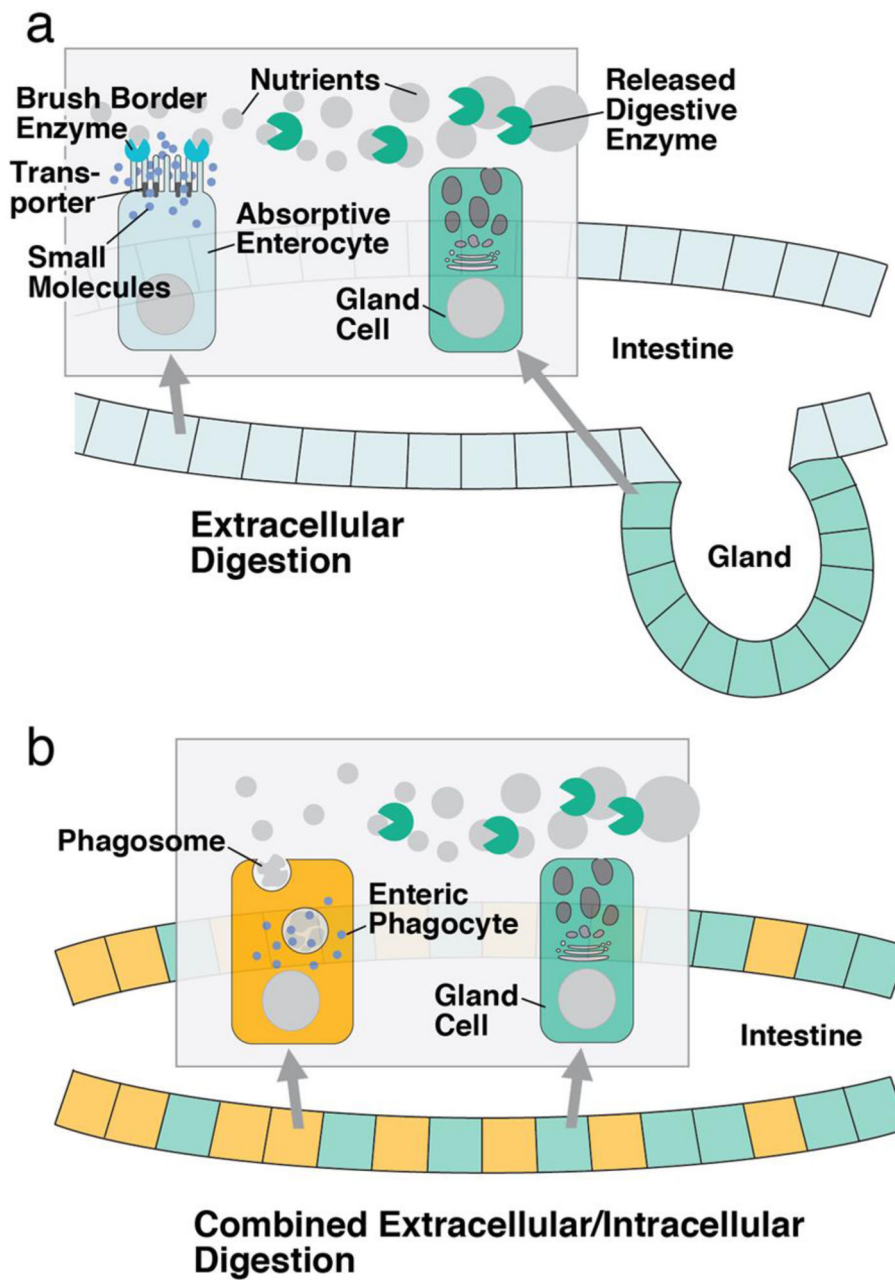


Figure 1. Schematic depiction of extracellular digestion in the vertebrate intestine and mixed extracellular/intracellular digestion observed for most invertebrate clades. **a** In extracellular digestion, enzymes secreted by specialized glands (e.g., pancreas) predigest food particles into macromolecules. These are then further broken down into small molecules by a different set of enzymes produced by the enterocytes. Small molecules are absorbed by diffusion or active transport. **b** In combined extracellular/intracellular digestion, gland cells distributed all over the intestine predigest food material into small particles and macromolecules. These are then taken up phagocytotically and digested intracellularly by enteric phagocytes.

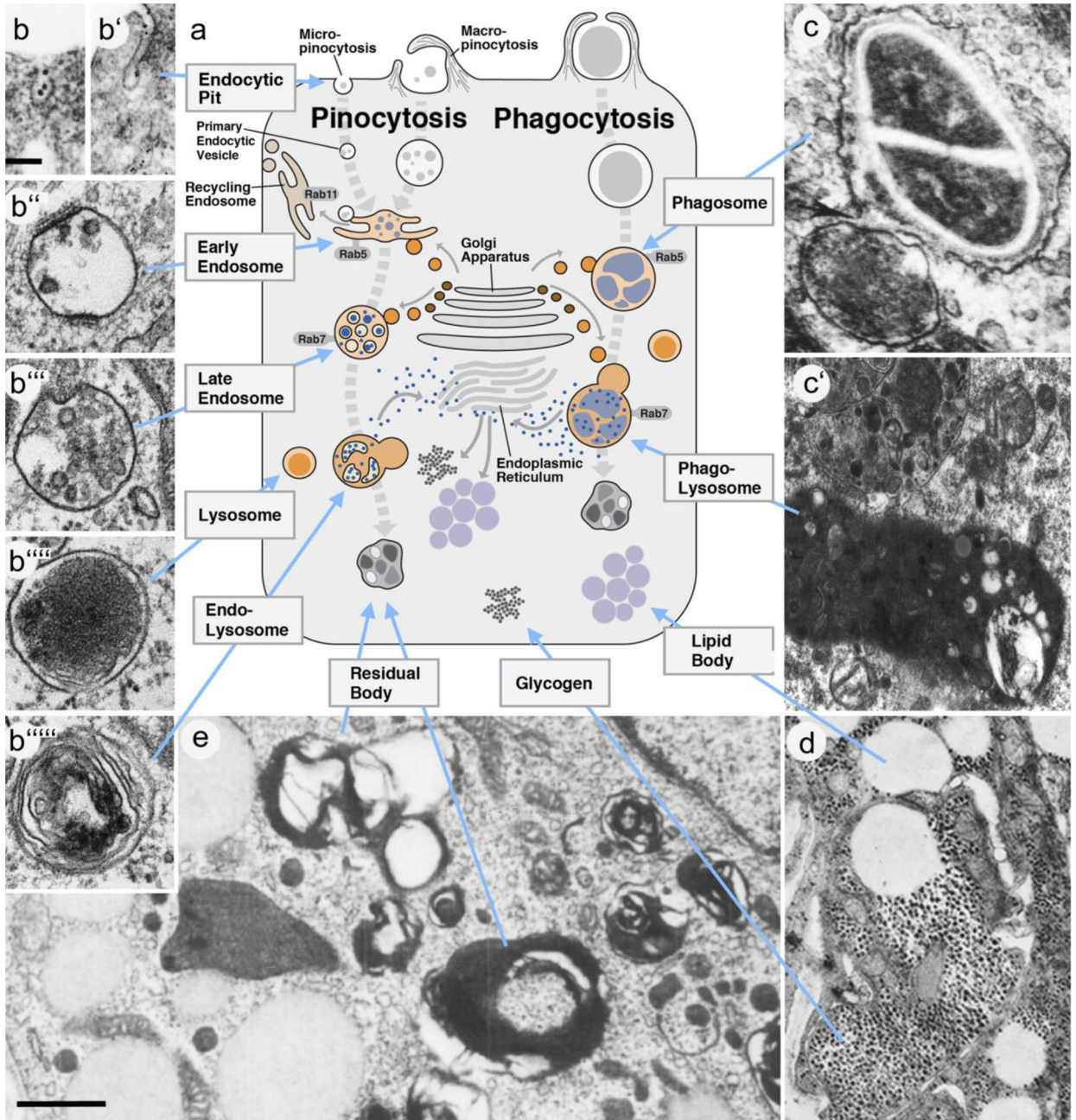


Figure 2. Structural aspects of the endosome-lysosome system involved in intracellular digestion. **a** Schematic diagram of phagocyte with its intracellular membrane bound compartments. Left side of diagram shows the endosomal-lysosomal pathway (thick gray hatched line) that degrades small food particles absorbed by pinocytosis. Compartments are identified by terms generally used in current textbooks and research literature. Right side of diagram depicts the phagosome-lysosome pathway followed by large food particles ingested by phagocytosis. Electron micrographs (**b-b''''''**, **c'-c''**, **d, e**) illustrate shape and texture of compartments shown in **a**. **b** Clathrin-coated endocytic pit. **b'** Non-clathrin-coated endocytic

pit. **b''** Early Endosome. **b'''** Late Endosome/Multivesicular body. **b''''** Lysosome. **b'''''** Endolysosome. **c** Phagosome containing bacteria. **c'** Two phagolysosomes; the one at bottom containing partially digested bacteria. **d** Lipid bodies and glycogen particles. **e** Residual bodies. **b**: from (Mayor and Pagano 2007) with permission; **b-b''''''**: from (Huotari and Helenius 2011) with permission; **c** from (Hard 1972) with permission; **d, e**: from (Del Conte 1979) with permission.

Scale bars: 100nm (b-d); 500nm (e).

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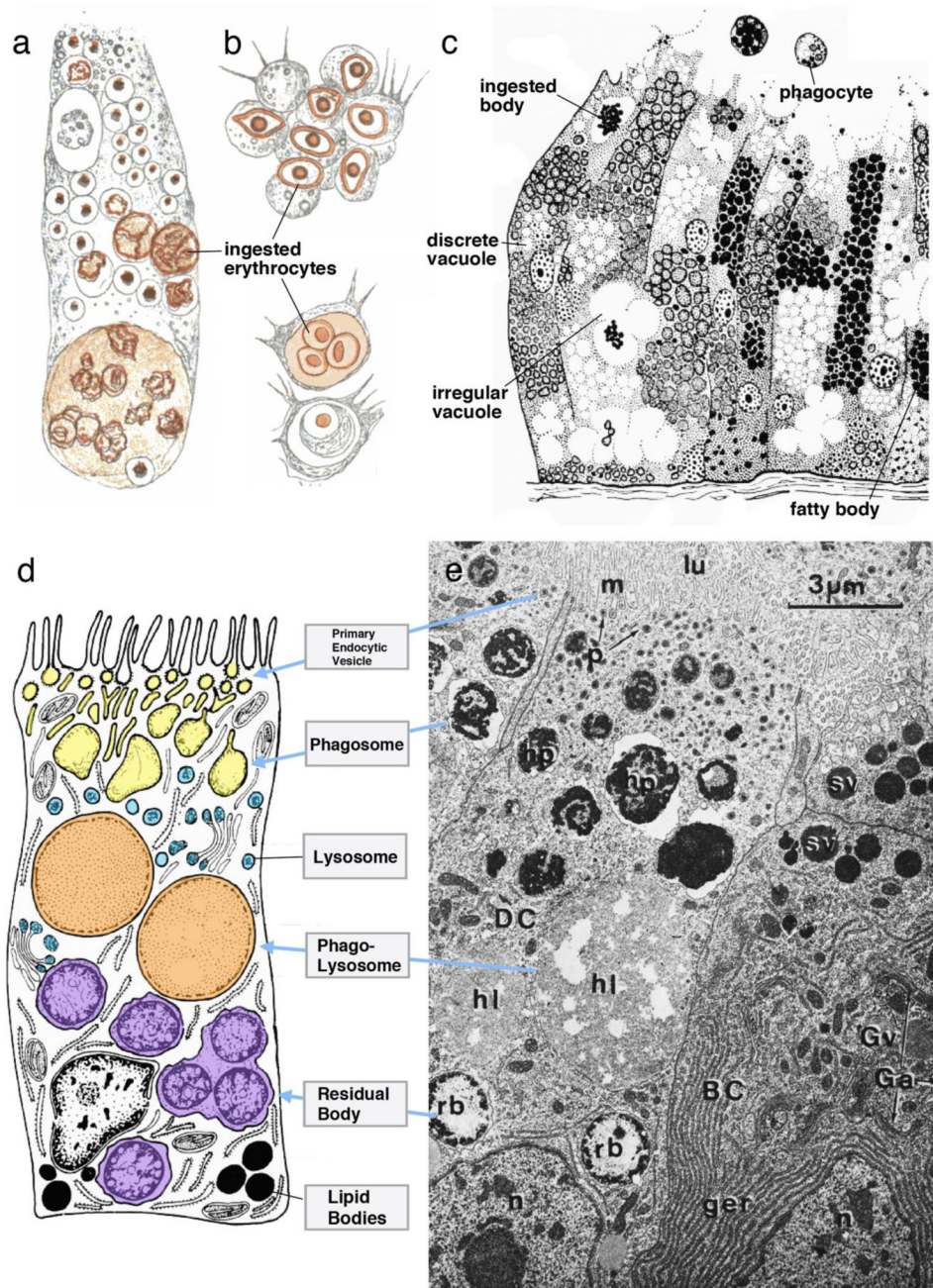


Figure 3. Structural aspects of enteric phagocytes. **a, b** Drawing of planarian enteric phagocyte (**a**) and invertebrate macrophages (**b**) phagocytosing erythrocytes (rendered in brown; from Metschnikoff, 1884). (**c**) Light microscopic appearance of epithelium of mollusk digestive gland, containing enteric phagocytes (left), glandular cells (right; from Millot, 1937, with permission). Terms used by author (“ingested body”, “discrete vacuole”, “irregular vacuole”, “fatty body” likely correspond to phagosome, phagolysosome, and lipid body, respectively). (**d, e**) Electron micrograph (**e**) and schematic drawing (**d**) of molluscan enteric phagocyte (from Owen 1972, with permission). Organelles of the phagosome/lysosome system,

depicted in **e**, are pointed out by blue arrows in **d**. Abbreviations: BC pyramidal basophilic cell (=zymogenic gland cell); DC digestive cell (=enteric phagocyte); Ga Golgi apparatus; ger granular endoplasmic reticulum; Gv Golgi vesicle; hl heterolysosome (=phagolysosome); hp heterophagosome (=phagosome); lu lumen of tubule; m microvilli; n nucleus; p pinosome (=primary endocytic vesicle); rb residual body; sv secretory vesicle.

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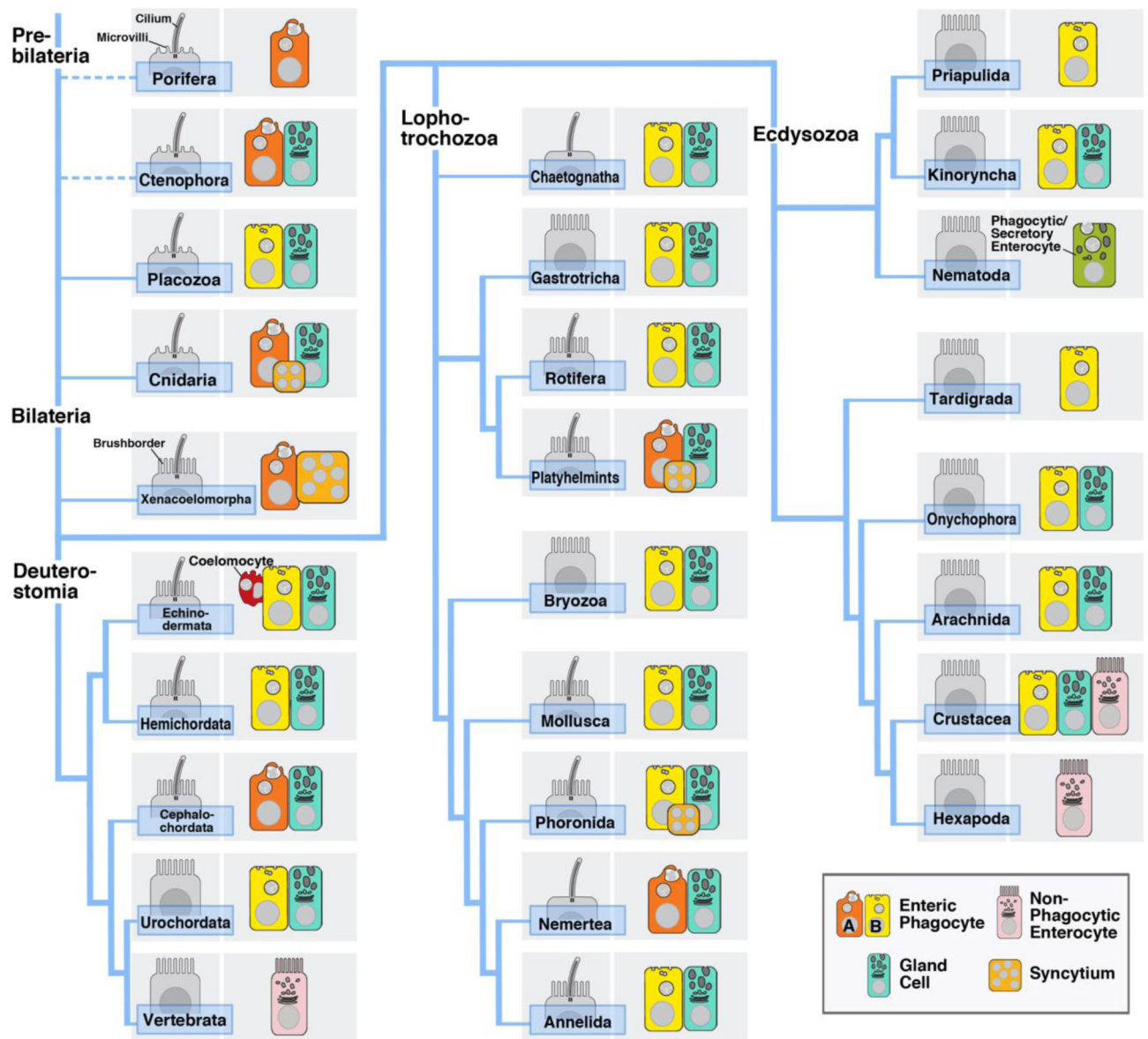


Figure 4. Digestive cell types in animals. Representative phyla are named (blue boxes) and ordered according to currently widely used phylogenetic trees (Peterson and Eernisse 2016; Kocot et al. 2017; Giribet and Edgecombe 2017) as indicated by blue lines. For each phylum, colored icons in right gray box symbolize major cell types forming the epithelium of the mid-intestine, as explained in framed box at upper right of figure. Nutrient absorption by enteric phagocytes described in the literature can be tentatively assigned to two types: Type A (orange) represents “canonical” phagocytosis, where entire cells or large particles are taken up; type B (yellow) describes a process where mostly smaller particles are taken up into endocytic vesicles that secondarily fuse into large phagosomes. Left grey boxes show different types of apical specializations (cilia, microvilli, dense microvilli organized as brushborder) of enteric phagocytes. For references of publications documenting digestive cell types, see Table 1.

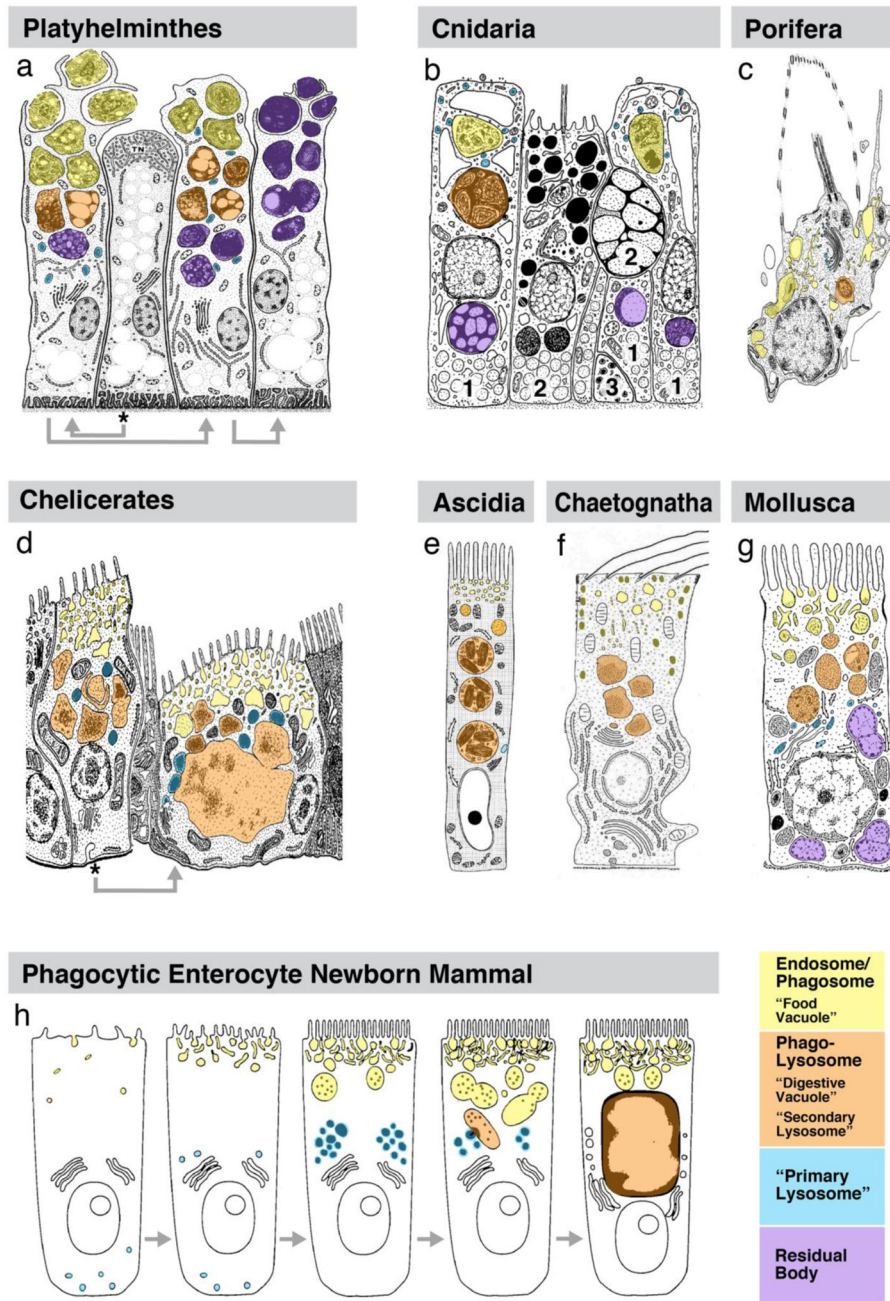


Figure 5. Schematic drawings of enteric phagocytes in representative phyla. Organelles of the phagosome/lysosome system are differentially rendered in yellow (apically located pits, endocytic vesicles, phagosomes), orange (phagolysosomes), blue (lysosomes), and violet (residual bodies; see bottom right of figure). **a** Phagocytes in platyhelminths (*Dugesia gonocephala*; from Garcia-Corrales and Gamo 1988, with permission). **b** Gastrodermal cell types in cnidarians (*Tripedalia cystophora*; from Chapman 1978, with permission). Numbers refer to phagocyte (1), secretory (spumous) cell (2), neurosecretory cell (3). **c** Choanocyte in sponges (*Ephydatia fluviatilis*; from Weissenfels 1976, with permission). Enteric phagocytes

in **(a-e)** perform “canonical” phagocytosis, whereby large food particles are surrounded by lamellipodia and drawn into large apical phagosomes. Panels of middle row (**d-g**) show different scenario, where food particles and macromolecules are endocytosed into small endocytic vesicles which, together with lysosomes, merge into larger phagolysosomes. This has been described, among others, for chelicerates (**d**; *Hyalomma asiaticum*; from Coons and Alberti 1999, with permission), ascidians (**e**; generic; from Burighel and Cloney 1997, with permission), chaetognaths (**f**; *Tenuisagitta setosa*; from Shinn 1997, with permission) and molluscs (**g**; *Mya arenaria*; from Pal 1972, with permission). **h** A similar type of endocytosis has also been described and illustrated for absorptive enterocytes of newborn mammals by (*Rattus norvegicus*; Wilson et al. 1991, with permission). Asterisks and grey arrows in panels **(a)** and **(d)** indicate time sequence, with cell marked by asterisk presenting morphology prior to food uptake, followed by later stages.

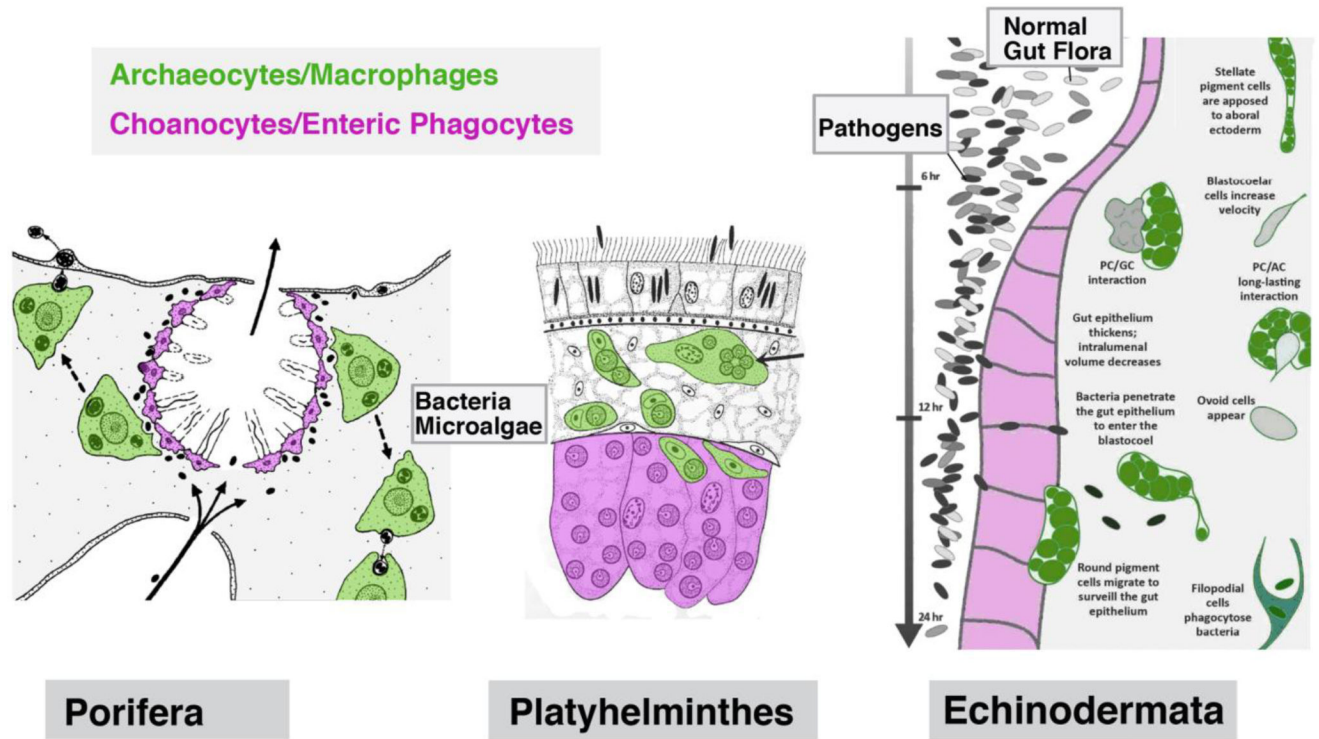


Figure 6. Functional interactions between enteric phagocytes and macrophages. Left: In sponges, food particles (bacteria, microalgae) are taken up by, and “swapped in between, stationary choanocytes (enteric phagocytes) and motile archaeocytes (macrophages; from Weissenfels 1976, with permission). Center: Even though not investigated at any length, transfer of algae taken up by enteric phagocytes to motile stromal cells has been documented for some flatworms (from Rieger 1991, with permission). Right: Coelomocytes (macrophages) in echinoderms are recruited towards the gastrodermis when pathogenic bacteria have entered the lumen of the gut (from Buckley and Rast 2017, with permission).

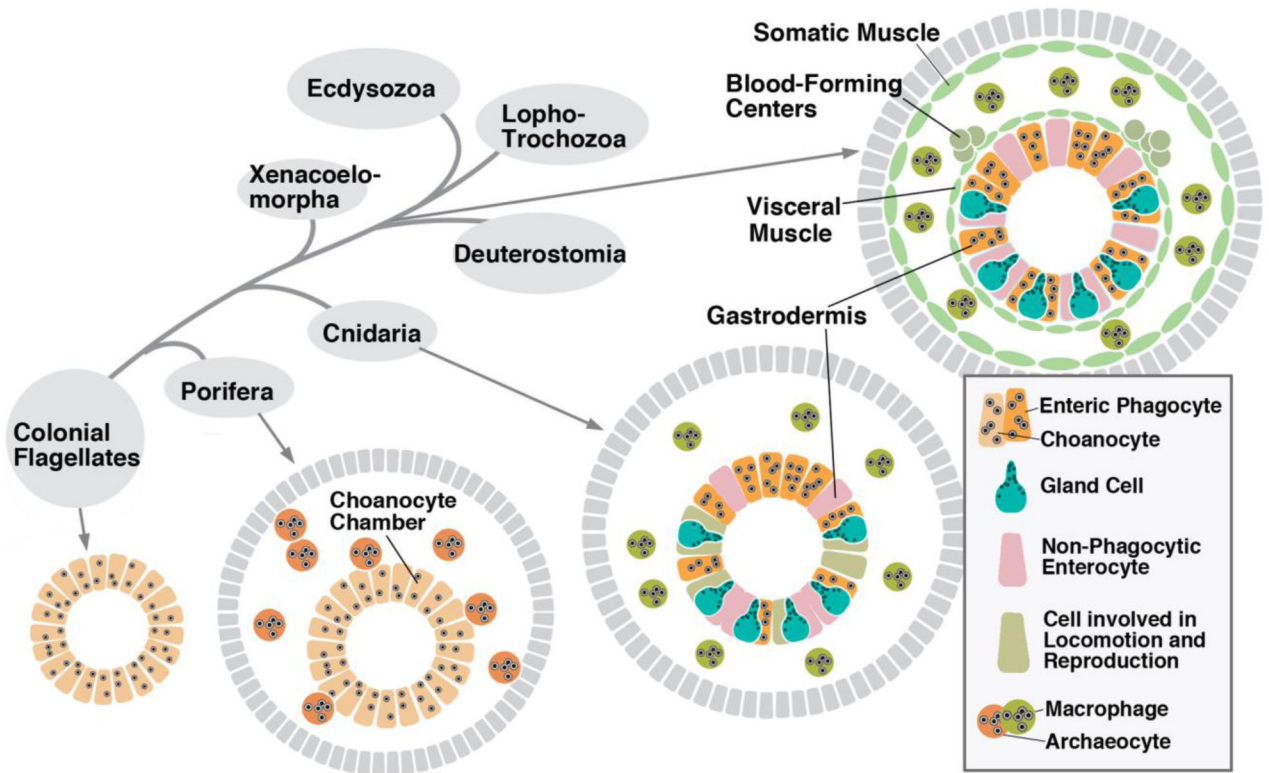


Figure 7. Distribution of enteric phagocytes and macrophages among animals. Simplistic phylogenetic tree is presented at upper left. Schematic cross sections of sponge (bottom center), cnidarian (bottom right) and coelomate bilaterian (top right) are grouped around the phylogenetic tree. Different cell types of endodermal digestive epithelium are represented in different shapes and colors, as explained in box at bottom right of figure. In sponges, motile macrophages and stationary phagocytes (choanocytes) may represent different stages in the same cell lineage; one stage is able to “transdifferentiate” into the other (Nakanishi et al. 2014; Sogabe et al. 2016). In cnidarians, the endodermal gastrodermis contains several differentiated cell types, among them enteric phagocytes. Motile macrophages (amoebocytes) with immune functions have been described (Menzel et al. 2015); it is possible that they are derived from the endoderm. In bilateria, mesodermal and endodermal cell lineages are separated early in development. Enteric phagocytes continue to develop from the endoderm; macrophages, along with other motile blood cells (hemocytes) are generated in specific hematopoietic centers within the mesoderm.

Table 1
Terminology used for enteric phagocytes and their organelles

Information tabulated here is taken from a comprehensive, yet non-exhaustive survey of published electron microscopic studies of the ultrastructure of invertebrate digestive systems. Columns A and B list animal clades. Column C indicates terms used for enteric phagocyte in corresponding animal clade. The term used for enteric phagocytes varies, often simply referring to the function (e.g., absorptive cell, digestive cell) or position (e.g., stomach cell, midgut cell) of the cell. Columns D-F show terms used for organelles associated with enteric phagocytes. The three columns reflect the position of the corresponding organelles along the apical-basal axis, with those referring to the apical membrane and its immediate vicinity placed in column D (yellow), the ones naming organelles in the supra/perinuclear space in column E (orange), and the ones referring to more basal structures in column F (purple). Based on this topology, structures referred to in column D would most likely correspond to pinocytotic/phagocytotic membrane specializations, primary endocytic vesicles/phagosomes and early endosomes; structures of column E to late endosomes, lysosomes and endolysosomes/phagolysosomes, and structures in column F to residual bodies and storage organelles. Column G indicates types of particles or cells used experimentally to ascertain phagocytosis. Literature is provided in column H.

Abbreviations: Ap. Apical; Cil. Ciliated; Col. Columnar; Endocyt. Endocytotic; Glyc. Glycogen particle; Incl. Inclusion; Intest. Intestinal; Multives. Multivesicular; Pinocyt. Pinocytotic; Prim. Primary; Resid. Residual; Sec. Secondary; Spher. Spherical; Vac. Vacuole; Ves. Vesicle

Clade	Subclade	Term for Cell	Membrane/ Apical	Supranuclear/ Perinuclear	Basal	Exp. Feeding	Reference
Porifera	Demospongiae	Choanocyte	Lamellipodia	Phagosome, Food Vac., Lysosome	Microvesicles	Bacteria	<i>1, 2</i>
	Demospongiae	Choanocyte	Small Ves.	Phagosome, Lysosome-like Ves.			<i>3</i>
	Calcarea	Choanocyte	Lamellipodia	Phagosome		Bead 1µm	<i>4</i>
	Homoscleromorpha	Choanocyte		Phagosome			<i>5</i>
Ctenophora		Digestive Cell	Ves.	Phagosome, Lysosome, Lysosome-like Body	Thorotrast	<i>6, 7</i>	
Cnidaria	Anthozoa	Phagocytic Cell	Pinocytic Ves.	Phagosome, Postphagosome, Lysosome	Wax Droplets, Glyc.		<i>8</i>
	Cubozoa	Absorptive Cell	Apical Discoideal Coated Ves.	Watery Vac., Phagosome, Lysosome	Lipid Droplets		<i>9</i>
	Hydrozoa	Gastrodermal Cell		Food Vac.			<i>10</i>
	Hydrozoa	Absorptive Cell	Discoideal Flattened Ves.			Ferritin	<i>11</i>
	Hydrozoa	Gastrodermal Cell	Flattened Coated Ves.		Large Vac.	Carbon part.	<i>12</i>
	Hydrozoa	Nutritive Phagocyte	Pinocyt. Ves., Microves.		Phagosome, Sec. Lysosome	Carbon part.	<i>13, 14</i>
	Echinodermata	Asteroidea	Enterocyte	Pinocyt. Vac.	Vac.		
Asteroidea		Choanocyte-like Cell	Coated Ves.	Phagosome, Multives. Body, Sec. Lysosome			<i>17</i>

Clade	Subclade	Term for Cell	Membrane/ Apical	Supranuclear/ Perinuclear	Basal	Exp. Feeding	Reference
Hemichordata	Ophiuroidea	Enterocyte	Pinocyt. Invagination, Ves.		Lipid Droplets, Glyc.		18
		Absorptive Phase Cell	Apical Coated Pit, Ves.	Heteromorphic Incl. Body, Digestive Vac.			19, 20
	Pterobranchia	Epithelial Cell	Apical Coated Pit, Coated Ves.	Multives. Body, Heteromorphic Vac. Body			21
Urochordata		Absorptive Cell	Coated Pit, Small Ves., Pinosome	Multives. Body, Vac., Sec. Lysosome	Lipid Droplet		22, 23, 24, 25
Cephalochordata		Vacuolated Cell	Micropinocyt. Ves.	Vac.			26
		Epithelial Cell	Coated Pit, Ves.	Lysosome, Granule	Lipid Droplet		27, 28
Chaetognatha		Phagocyte		Lysosome, Phagosome		Algae	29
		Absorptive Cell	Pinocyt. Ves.	Lysosome-like Body			30, 31
Gastrotricha		Absorptive Vac. Cell	Coated Pit, Endocyt. Ves.	Vac., Endolysosomal Compartment			32
		Absorptive Cell	Pinocyt. Ves., Small Vac.	Lysosome-like Body, Sec. Lysosome	Glyc.		33, 34
Rotifera		Stomach Cell	Caveolae, Coated Ves.	Food Vac.		Thorotrast	35, 36
Platyhelminthes		Stomach Cell		Food Vac., Incl. Body (=Lysosome)			37
		Col. Phagocytic Cell	Pinocyt. Ves.	Phagosome, Lysosome, Sec. Lysosome	Lipid Droplet, Glyc.		38
	Tricladida	Phagocytic Cell	Pseudopodia, Food Vac.	Lysosome, Digestive Vac.	Resid. Body, Lipid Droplet, Glyc.		39, 40, 41
	Dalatyphloplanida	Gastrodermal Cell	Micropinocyt. Ves.	Phagosome, Vac.	Lipid Globule		42
	Catenulida	Phagocyte	Coated Endocyt. Ves.	Vac., Lysosome	Resid. Body, Lipid Incl.	Latex Bead	43
Bryozoa	Macrostomida	Phagocytic Cell	Food Vac.	Food Vac., Lysosome	Lipid Droplet		44, 45
		Absorpt. Acid. Cell	Endocyt. Ves., Prim. Lysosome	Digestive Vac. (Sec. Lysosome)			46
Phoronida		Cil. Epithelial Cell	Pinocyt. Ves., Ap. Spher. Granule	Digestive Vac., Ves. Containing Nutrient	Lipid Body		47, 48
Nemertinea		Cil. Gastrodermal Cell	Oval Body, Food Vac.	Lysosome			49, 50
Annelida	Polychaeta	Absorptive Cell	Dense Body	Lysosome	Lipid Droplet, Glyc.		51, 52

Clade	Subclade	Term for Cell	Membrane/ Apical	Supranuclear/ Perinuclear	Basal	Exp. Feeding	Reference
Mollusca	Hirudinea	Intest. Epithelial Cell	Caveolae, Apical Dense Body	Vac.	Lipid Inclusion		53, 54
	Gastropoda	Digestive Cell	Endocyt. Ves., Multives. Body	Lysosome, Heterolysosome, Large Vac.	Resid. Body, Lipid Droplet, Glyc.		55, 56, 57
	Gastropoda	Digestive Cell	Small Coated Ves.	Large Vac.		Thorotrast	58
	Bivalvia	Digestive Cell	Microves., Pinoosome, Phagosome	Macroves. (Phagolysosome)	Resid. Body, Lipid-like Droplet	Graphite	59, 60, 61
	Bivalvia	Absorptive Cell	Pino/ Macropinosome, Phagosome	Lysosome, Heterolysosome	Resid. Body	Nanoparticle	62
	Cephalopoda	Digestive Cell	Pinocytotic Ves.	Digestive Vac.	Brown-Body Vac., Lipid Droplet		63
Priapulida		Epithelial Cell	Ves., Lysosome	Digestive Vac.	Lipid Droplet, Glyc. Particles		64, 65
Kinorhyncha		Epithelial Cell		Digestive Vac.			66, 67
Nematoda		Intest. Epithelial Cell	Pinocyt. Ves., Phagosome	Prim. Lysosome, Phagolysosome	Pigment Gran., Lipid Droplet, Glyc.	Hemoglobin	68, 69, 70
		Intest. Cell	Endosome, Phagosome	Lysosome, Sec. Lysosome	Lipofuscin Granule, Telolysosome		71
Tardigrada		Digestive Cell	Coated Pit, Ves., Multives. Body	Digestive Vac., Lysosome, Sec. Lysosome	Spher. Incl., Lipid Droplet, Glyc.		72, 73, 74
Onychophora		Absorptive Cell	Ves.	Membrane-bound Incl.	Lipid Droplet, Glyc.		75
Crustacea	Copepoda	Midgut Cell	Pinocyt. Vesicle	Lysosome, Heterophagosome			76
	Isopoda	Digestive Cell	Pinocyt. Vesicle	Lysosome-like Body, Vac.	Lipid Droplet, Glyc.		77
	Malacostraca	Digestive Cell	Vesicle	Phagosome, Lysosome-like Granule	Lipid Droplet		78
	Decapoda	Digestive (B) Cell	Pinocyt. Vesicle	Subapical Vac., Supranuclear Vac.			79, 80
Chelicerata	Scorpiones	Digestive Cell	Pinocyt. Vesicle, Channel Vesicle	Lysosome-like Organelle, Brown Body Vac.	Glyc., Lipidic Inclusions	Colloid. Gold	81
	Acariformes	Digestive Cell	Pinocyt. Vesicle, Phagosome	Lysosome, Sec. Lysosome	Resid. Body, Lipid Droplet		82, 83, 84

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¹³McNeil 1981;

¹⁴Fitt and Trench 1983;

¹⁵Jangoux 1972;

¹⁶Chia and Koss 1991;

¹⁷Martinez et al 1991;

¹⁸Byrne 1994;

¹⁹Benito and Pardos 1997;

²⁰Welsch and Dilly 1980;

²¹Dilly et al 1986;

²²Thomas 1970;

²³Burighel and Milanesi 1973;

²⁴Burighel and Milanesi 1975;

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²⁷Ruppert 1997;

²⁸Welsch and Storch 1969;

²⁹He et al. 2018;

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- ⁶³ Boucaud-Camou and Yim 2009;
- ⁶⁴ Storch et al. 1989;
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- ⁷²Greven 1976;
- ⁷³Dewel et al. 1993;
- ⁷⁴Biserova and Mustafina, 2015;
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- ⁷⁸Loizzi 1971;
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- ⁸¹Goyffon and Martoja 1983;
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