Developmental Cell
Review

Diffusion Barriers, Mechanical Forces, and the Biophysics of Phagocytosis

Philip P. Ostrowski,[1,2] Sergio Grinstein,[1,2,3,*] and Spencer A. Freeman[1]
[1]Program in Cell Biology, Peter Gilgan Centre for Research & Learning, Hospital for Sick Children, 686 Bay Street, Toronto, ON M5G 0A4, Canada
[2]Department of Biochemistry, University of Toronto, Toronto, ON M5S 1A8, Canada
[3]Keenan Research Centre of the Li Ka Shing Knowledge Institute, St. Michael's Hospital, 290 Victoria Street, Toronto, ON M5C 1N8, Canada
*Correspondence: sergio.grinstein@sickkids.ca
http://dx.doi.org/10.1016/j.devcel.2016.06.023

Phagocytes recognize and eliminate pathogens, alert other tissues of impending threats, and provide a link between innate and adaptive immunity. They also maintain tissue homeostasis, consuming dead cells without causing alarm. The receptor engagement, signal transduction, and cytoskeletal rearrangements underlying phagocytosis are paradigmatic of other immune responses and bear similarities to macropinocytosis and cell migration. We discuss how the glycocalyx restricts access to phagocytic receptors, the processes that enable receptor engagement and clustering, and the remodeling of the actin cytoskeleton that controls the mobility of membrane proteins and lipids and provides the mechanical force propelling the phagocyte membrane toward and around the phagocytic prey.

Introduction

2016 marks the centenary of the death of Elie Metchnikoff, whose incisive intelligence and foresight are best illustrated by his elegant studies of phagocytosis. He recognized that rather than being merely a vestige of a nutrient-acquisition system in single-celled organisms, phagocytosis evolved to become a critical defense mechanism against pathogens. This realization was at the heart of his Nobel-prize-winning research on cell-mediated immunity. But even Metchnikoff could not foresee the ever-growing number of roles attributed to phagocytes, ranging from tissue development and homeostasis, to wound healing and promotion of atherosclerosis and metastasis.

Many cell types can perform phagocytosis, but professional phagocytes specialize and excel at engulfing and eliminating their targets. Of these, macrophages have been studied in greatest detail because they are more amenable to molecular manipulation, and generally more forgiving than are neutrophils and dendritic cells. For reasons of space, this review centers on the phagocytic function of macrophages, paying particular attention to the cytoskeleton and its interplay with the glycocalyx, the pericellular coat that surrounds virtually every animal cell. The glycocalyx, which is composed of membrane glycoproteins and glycolipids, as well as adherent polysaccharides, forms a barrier of varying density and length that can curtail access of macromolecules and particulate material to the surface of the cell; its role in phagocyte biology has been sorely ignored. We explore the conditions that prime macrophages for phagocytosis, the first contacts between the phagocytes and their prey, and the events that orchestrate remodeling of the cytoskeleton for successful particle engulfment. The complexity and versatility of phagocytosis, as well as the enormous gaps in our understanding of the process will become evident in the following text. For these reasons, together with the fact that it is highly conserved and essential for survival, we believe that phagocytosis is poised to remain a cornerstone of discovery in cell biology for the next 100 years.

First Contacts between Phagocytes and Phagocytic Prey

Mature macrophages are often lodged within a defined anatomical niche, where they perform tissue-specific functions. Such tissue-resident macrophages are largely non-migratory, especially compared with neutrophils and monocytes. They are, however, well positioned within their respective niches to encounter their phagocytic prey, whether pathogens, dead cells, or debris. Kupffer cells, the largest population of tissue-resident macrophages, reside in the lumen of hepatic sinusoids, poised to capture dangerous blood-borne pathogens (Bilzer et al., 2006). Microglia are widely and homogeneously distributed in the brain to assess their individual territory for dead/dying neurons or plaque-forming complexes (Nimmerjahn et al., 2005). Single bone-resident macrophages form the centers of erythroblastic islands (the sites where red blood cells are generated), capturing the nuclei extruded by erythroblasts as they become erythrocytes (Chasis and Mohandas, 2008). Phagocytes resident in the lymphatic system persistently sample particles carried by the lymph (Batista and Harwood, 2009; Gerner et al., 2015; Kuan et al., 2015).

Initial contact between phagocytes and their prey could in principle be mediated by Brownian-driven collisions, or by the directed motion of particulates propelled by flagella, or by the flow of blood or lymph. However, complete reliance on such random events would seem dangerously insufficient. Instead, contact frequently involves the extension of active membrane protrusions by the phagocytes (Figure 1A). Filopodia and broad membrane ruffles attach to pathogens before they are engulfed (Vonna et al., 2007), and cells unable to form these structures fail to engage stationary phagocytic prey (Flannagan et al., 2010). Membrane protrusions allow phagocytes to reach spaces their cell bodies cannot access, increasing the radius of the zone they scan (Nimmerjahn et al., 2005). In addition to reaching targets, actin-driven membrane extensions exert force, which may play an unappreciated role in accessing receptors

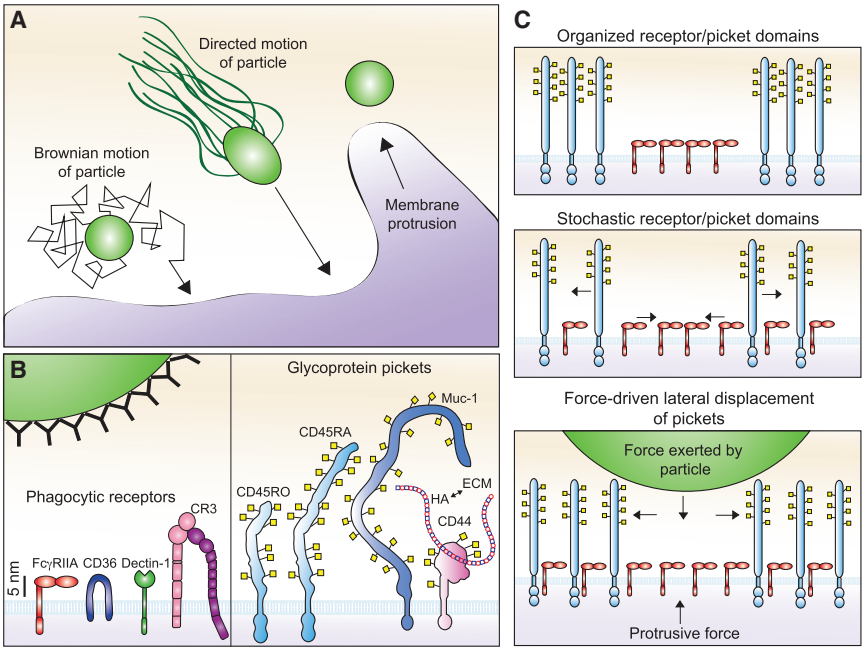

Figure 1. First Contact between Phagocytes and Their Prey

(A) Phagocytic prey can strike the phagocyte surface by Brownian-driven collisions or as a result of directed motion propelled by flagella or by fluid flow. In addition, phagocytes can extend membrane protrusions to engage their targets.

(B) Glycoprotein pickets extend further than phagocytic receptors, presenting a steric barrier to receptor engagement.

(C) Three possible mechanisms for the prey to gain access to phagocytic receptors to initiate the activation process.

concealed by a thick layer of glycocalyx. The protrusive force of filopodia, membrane ruffles, and podosomes/invadopodia, estimated to be in the range of 3–100 pN (Cojoc et al., 2007; Labernadie et al., 2014), may thus facilitate the first engagement of phagocytic targets. The role of such forces in overcoming physical and steric barriers to the access of phagocytic receptors is discussed below.

How Phagocytic Receptors Contact Their Ligands

Macrophages are challenged by a diverse array of phagocytic targets and ligands, necessitating the expression of a vast repertoire of receptors. With the exception of a few scavenger receptors (Canton et al., 2013), phagocytic receptors, including the prototypical Fc receptors and the fungal receptor Dectin-1, are all short molecules, protruding only ≈5 nm from the outer leaflet of the bilayer (Figure 1B). This is in sharp contrast with the much longer, often rigid transmembrane glycoproteins prevalent throughout the membrane (Figure 1C). It was Timothy Springer (Springer, 1990) who first realized that long and rigid features of the leukocyte glycocalyx could shield short receptors. Components of the glycocalyx, such as CD45 and CD148 (transmembrane phosphatases), transmembrane mucins (proteins that are highly glycosylated through GalNAc O-linkages at threonine and serine residues), and the pericellular matrix generated by CD44 and hyaluronan, can reduce ligand access and oppose sustained contact by decreasing overall entropy when compressed. One such example, the transmembrane phosphatase CD45, has served as an archetypal marker for leukocytes in part because of its abundance. In T lymphocytes, CD45 outnumbers T cell receptors (TCRs) by at least a 3 to 1 margin (Chang et al., 2016). The ectodomain of CD45 is rigid and even its shortest isoform extends ≈20 nm, presenting a steric barrier perpendicular to the plasma membrane for the engagement of short phagocytic receptors (Figure 1B).

If a tall, dense layer of glycocalyx exists around the cell, how do shorter receptors ever engage particle-associated ligands to initiate the activation process? We can envisage three possibilities (Figure 1C). First, the layer of glycocalyx may not be continuous; areas where some receptors are segregated and exposed may exist constitutively. There is evidence obtained by super-resolution and electron microscopy for the formation of protein domains or islands in resting cells, and fine atomic force microscopy measurements have unveiled heterogeneity of glycocalyx height in endothelial cells (Oberleithner et al., 2011). By confocal microscopy, however, we have found that the regions of the macrophage membrane that make first contact with target particles are, in fact, richly endowed in CD44 and CD45 (S.F. and S.G., unpublished data), while others have observed a homogeneous distribution of the glycocalyx in non-adherent regions of tumor cells (Paszek et al., 2014).

A second possibility is that the stochastic motion of the taller molecules may momentarily expose the shorter receptors, enabling them to contact the target particle. We regard this scenario as unlikely, because comparatively large exposed areas would need to be generated to allow access to sizable, rigid particles. Third, and most attractive, we speculate that mechanical force exerted either by the approaching particle and/or by the phagocyte itself as it actively extends protrusions may suffice to displace the glycocalyx laterally, bringing the ligands in close apposition with the receptors. Indeed, applying exogenous force on cells or increasing the rigidity of substrates on which they are grown leads to the displacement of large glycoproteins from adhesive contacts (Paszek et al., 2014; Van Goethem et al., 2011), lending support to this idea. Nevertheless, the three preceding possibilities are not mutually exclusive and require more thorough experimental testing.

Molecular Diffusion of Glycoproteins in the Plasma Membrane

The segregation of the glycocalyx from receptors to initiate signaling and the subsequent clustering of phagocytic receptors require lateral diffusion of glycoproteins and receptors in the plane of the plasma membrane. Such diffusion can meet with considerable physical obstacles; the glycocalyx components as well as the receptors could be tethered or may be confined