Abstract

Phagocytosis is an phylogenetically conserved mechanism utilized by many cells to ingest microbial pathogens and apoptotic or necrotic corpses. Recent studies have demonstrated that phagocytosis serves to initiate immunity mediated by both Class I and Class II MHC. Depending on the identity of the specific phagocytic receptor involved, phagocytosis can either enhance or suppress inflammation. Dysregulation of phagocytosis can lead to alterations in the immune response and may contribute to autoimmunity. Harnessing the phagocytic capacity of antigen presenting cells may ultimately lead to exploitation of phagocytosis as a therapeutic modality in intractable diseases, such as advanced cancer.

Phagocytosis is the process by which leukocytes and other cells ingest particulate ligands whose size exceeds about 1 μm. This phylogenetically ancient cellular event is critical for both innate and acquired immunity. By ingesting microbial pathogens, phagocytic leukocytes accomplish two essential immune functions. First, they initiate a microbial death pathway. They target ingested pathogens to degradative organelles, such as lysosomes and to vesicles containing components of the phagocyte oxidase complex. Second, phagocytic leukocytes, particularly dendritic cells (DCs), utilize phagocytosis to direct antigens to both MHC I and II compartments. Thus, phagocytosis serves a dual role as an effector of innate immunity and an initiator of acquired immunity.

Diversity of Phagocytic Receptors

Many receptors are capable of mediating phagocytosis (Table 1). These receptors can be broadly defined as those that recognize epitopes on the surfaces of unmodified bacteria and fungi (nonopsonic phagocytosis) or those that recognize components derived from the host (opsonic phagocytosis). Examples of the latter include receptors for the Fc portion of IgG (FcyRs) and receptors that recognize various components of complement (e.g., Complement Receptor 3). It is important to distinguish between those receptors that participate solely in binding of the phagocytic target with receptors that are actually coupled to the transmembrane signaling machinery of phagocytosis. For example, many members of the Scavenger Receptor superfamily are “pattern recognition receptors,” which bind ligands, such as peptidoglycan, present on the surfaces of bacteria and fungi. However, most of these receptors have not been shown to trigger distinct transmembrane signals; rather, they facilitate the recruitment of other receptors that directly engage the phagocytic machinery.
Table 1. Examples of receptors in mammalian cells that promote phagocytosis$^a$

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Cell Type</th>
<th>Target</th>
<th>Ligand</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcγRI</td>
<td>Gran, Mo, MΦ, DCs, Mast, Pits</td>
<td>IgG-coated pathogens</td>
<td>Fc port ion of IgG</td>
<td>many</td>
</tr>
<tr>
<td>FcγRIIA</td>
<td></td>
<td>Complement-opsonized bacteria and fungi</td>
<td>C3b, C4b Mannan-binding lectin</td>
<td>84</td>
</tr>
<tr>
<td>FcγRIII</td>
<td></td>
<td>Complement-opsonized bacteria and fungi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR1 (CD35)</td>
<td>PMN, Mo, MF</td>
<td>Gram-negative bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR3 (CD11b/CD18; αμβ2; Mac1)</td>
<td>PMN, Mo, MΦ, myeloid DCs</td>
<td>Complement-opsonized bacteria and fungi</td>
<td>C3bi, C3d</td>
<td>85</td>
</tr>
<tr>
<td>CR4 (CD11c/CD18; p150, 95)</td>
<td>MΦ, DC</td>
<td>Gram-negative bacteria</td>
<td>LPS</td>
<td></td>
</tr>
<tr>
<td>CD48</td>
<td>Mast</td>
<td>Enterobacteria</td>
<td>FimH</td>
<td>87</td>
</tr>
<tr>
<td>Dectin-1</td>
<td>MΦ</td>
<td>P. carinii, C. albicans</td>
<td>Mannosyl/fucosyl residues</td>
<td>88</td>
</tr>
<tr>
<td>Scavenger receptor A/II</td>
<td>Apoptotic lymphocytes</td>
<td></td>
<td>?PS</td>
<td>89-91</td>
</tr>
<tr>
<td>Scavenger receptor B/I</td>
<td>Sertoli cells, Thymic Epi</td>
<td>Apoptotic cells</td>
<td>Mannosyl/fucosyl residues</td>
<td></td>
</tr>
<tr>
<td>MARCO</td>
<td>MΦ</td>
<td>E. coli, S. aureus</td>
<td></td>
<td>94</td>
</tr>
<tr>
<td>Mer</td>
<td>MΦ</td>
<td>Apoptotic thymocytes</td>
<td>?Gas6/PS</td>
<td>95</td>
</tr>
<tr>
<td>PSR</td>
<td>Many</td>
<td>Apoptotic cells</td>
<td>PS</td>
<td>96</td>
</tr>
<tr>
<td>CD36</td>
<td>MΦ</td>
<td>Apoptotic PMN</td>
<td>PS/Thrombospondin</td>
<td>97,98</td>
</tr>
<tr>
<td>CD14</td>
<td>MΦ</td>
<td>P. aeruginosa</td>
<td>?LPS</td>
<td>99,100</td>
</tr>
<tr>
<td>β1 integrins</td>
<td>Many</td>
<td>Yersinia</td>
<td>Invasin</td>
<td>101</td>
</tr>
<tr>
<td>α4β7</td>
<td>MΦ</td>
<td>Apoptotic cells</td>
<td>?Thrombospondin</td>
<td>97,102</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Epi</td>
<td>Listeria</td>
<td>InLA</td>
<td>34</td>
</tr>
<tr>
<td>Met</td>
<td>Epi</td>
<td>Listeria</td>
<td>InLB</td>
<td>31</td>
</tr>
</tbody>
</table>

$^a$Specific inhibition of binding by these receptors correlates with inhibition of phagocytosis. However, with some notable exceptions (e.g., FcγRIIA and the macrophage mannose receptor), it is possible that the indicated receptor serves to enhance ligand binding, rather than to participate directly in the ingestion process;\( ^b \)DC = dendritic cells; Epi = epithelial cells; Leuk = leukocytes; Mast = mast cells; Mο = monocytes; MΦ = macrophages; PMN = polymorphonuclear leukocytes; PS = phosphatidylinerine

Mechanisms of Phagocytic Signaling

The signaling mechanisms of phagocytosis are well-understood for only a handful of receptors. FcγRs signal by recruiting an array of tyrosine kinases in the vicinity of the ligated receptors. FcγR ligand binding receptors or their subunits contain immunoreceptor tyrosine-based activating motif (ITAMs) in their cytosolic domains. These motifs become phosphorylated by members of the Src family, which serve as high-affinity binding sites for SH2 domains of Syk, a tyrosine kinase that is expressed predominantly in hematopoietic cells. Although Syk is clearly required for phagocytosis, the identity of further downstream signals that are critical for phagocytosis are less certain. Phospholipid kinases (phosphatidylinositol 3-kinase; PI 3-kinase$^9-11$ and phosphatidylinositol-4-phosphate 5-kinase$^{12}$) are clearly involved
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as are various serine/threonine protein kinases (e.g., MEK1/2 and/or ERK, in the case of neutrophils) and PKC. PLA2 and PLD are also activated and believed to participate in the phagocytic process. The former may participate in vesicle trafficking during phagocytosis as well as contribute to the production of leukotrienes that amplify the phagocytic signal. Ultimately, early phagocytic signaling events culminate in net actin assembly and pseudopod extension. Actin assembly during phagocytosis is mediated by one or more Rho family GTPases. Other GTPases may participate in actin polymerization and/or vesicle trafficking, such as ARF6. In contrast, PI 3-kinase regulates pseudopod extension, in part by recruiting intracellular pools of latent phagosomal membrane and in part by recruiting PH domain-containing proteins, such as myosin-X, to the phagosome.

The occurrence of ITAMs in other phagocytic receptors suggests strongly that the paradigm of phagocytic signaling utilized by FcγRs is likely to be a general one. For example, Dectin-1, a lectin that recognizes mannosyl/fucosyl residues on fungi, and CEACAM3, a receptor that recognizes Neisseria, Moraxella, and Haemophilus species contain functional ITAMs in their cytosolic domains.

Fate of Engulfed Pathogens—Us vs. Them

The initial host response to most bacterial and fungal pathogens is phagocytosis. The particular route of entry is a function of the nature of the pathogen being ingested and identity of the host cell receptors engaging the pathogen. For example, internalization of Listeria is mediated by the adhesins InlA, which binds to E-cadherin on host epithelia, and InlB, which binds to the Met tyrosine kinase and to gC1q-R on host cells. E-cadherin-mediated entry requires participation of catenins and Met-dependent signaling induces activation of PI 3-kinase. For Yersinia, recognition of invasin on the bacterial surface is mediated by β2 integrins and bacterial uptake requires the participation of Src-family tyrosine kinases and focal adhesion kinase.

Phagocytosis can be a highly localized event (e.g., phagocytosis mediated by "zippers") or can be partially delocalized by virtue of a diffusible mediator. Several pathogens, such as Salmonella or Shigella, stimulate a "trigger" mechanism of invasion, inducing the assembly of actin in the host cell in the vicinity of where the bacteria interact with the host. Using a Type III secretion system, Salmonella injects SopE, a protein with guanine nucleotide exchange factor for Cdc42 and Rac, into host cells. In contrast, some pathogens, such as Haemophilus ducreyi and Yersinia, use different strategies to evade phagocytosis. Yersinia secretes YopH, a tyrosine phosphatase that dephosphorylates the focal adhesion protein, Cas, as well as other potential tyrosine kinase substrates. Another secreted product of Yersinia, YopE, is a RhoGAP.

Evolutionary pressure results in genetically stable adaptations by bacteria that serve to compromise the host. Many pathogens evade killing by inducing a delay in phagosome maturation. This can result in exclusion of active lysosomal enzymes or components of the NADPH-oxidase-containing vesicles from the phagosomes. Among the survival strategies employed by Mycobacterium tuberculosis, for example, is the suppression of calcium signaling, which contributes to evasion of lysosome fusion by the Mycobacterium-containing phagosome. Some of the phagosome maturation arrest activity resides in glycosylated lipids derived from the cell wall of M. tuberculosis. Other pathogens, such as Legionella, avoid maturation at early stages of phagosome biogenesis. The Legionella phagocytic vacuole is highly specialized: it does not fuse with lysosomes, fails to acidify, and intercepts vesicular traffic from ER exit sites to create an organelle that permits intracellular replication.

Phagocytosis and the MHC Class II Pathway

Once ingested, microbes that reside in phagocytic vacuoles find themselves in an increasingly hostile environment. Phagosomes undergo a maturation process, beginning with
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recruitment of Rab-5-positive early endosomes, followed by fusion with Rab-7-positive late endosomes. This is accompanied by further maturation and fusion with lysosomes. The capacity of lysosomes to degrade proteins is under developmental control. Mature dendritic cells demonstrate an enhanced capacity to degrade antigen, which correlates with a greater acidification of lysosomes and enhanced lysosomal vacuolar-ATPase activity. Following degradation and loading onto MHC Class II, mature DCs generate tubules from lysosomal compartments, which fuse directly with the plasma membrane.

Phagocytosis and the MHC Class I Pathway-Intracellular Pathogens and Tumors

In the past few years, much progress has been made to explain the cellular basis for the phenomenon termed “cross-priming,” first described by Bevan in 1976. This method of antigen presentation relies on the phagocytosis of an apoptotic cell (e.g., induced by viral infection) and the presentation of phagocytically-derived viral antigens onto MHC Class I. Previously, it had been thought that loading onto MHC Class I occurs only following loading of endogenous antigen from the cytosol to the ER. In contrast, the “phagosome-to-cytosol” pathway of antigen processing involves translocation of peptides or proteins from within the phagosome to the cytoplasm. There is precedence for proteins crossing membrane barriers; this is the principal mechanism for protein import into mitochondria, for example. How do phagosomal membranes become modified to accomplish a similar task? The observation that ER membrane has the capacity to fuse with phagosomal membrane offers one potential mechanism for this mode of antigen presentation. Sec61, an ER-derived protein translocation channel, was observed to become incorporated into phagosomes of dendritic cells. Because this protein is capable of “reverse transport” of proteins (in the opposite direction of its recognized ability to translocate nascent chains into the ER), it is possible that Sec61 provides the means by which proteins translocate across the membrane of the “phago-ER-some” into the cytosol. Once in the cytosol, the protein can be targeted to the proteasome for degradation and further MHC Class I processing by the conventional TAP-dependent pathway.

Cross-priming may be essential for immunity to viruses and other intracellular pathogens. Using a mouse model of viral immunity, Rock and colleagues demonstrated that virally infected nonhematopoietic cells are unable to stimulate primary CTL-mediated immunity directly. Instead, bone-marrow-derived cells are required as antigen-presenting cells to initiate anti-viral CTL responses. From a teleological standpoint this makes sense. Viruses that typically do not infect hematopoietic cells fail to gain entrance to secondary lymphoid organs. Therefore, they are unlikely to serve as efficient antigen-presenting cells in a primary immune response. However, this interpretation has been called into question. In one recent study, poliovirus in naturally nonpermissive murine APCs acquired viral RNA in vivo independently of the cellular virus receptor. The polioviral RNA initiated neosynthesis of viral antigen sufficient to prime CTLs in vivo. It thus remains an open question as to the relative importance of the endogenous pathway and cross-priming in MHC Class I-restricted immunity in vivo.

Cross-priming may be critical for tumor immunity. Although this pathway has been demonstrated in a variety of mononuclear phagocytes, it appears that dendritic cells are the most potent APCs in stimulating phagosome-derived MHC Class I-restricted CD8+ CTLs. Many strategies for using dendritic cells loaded with tumor-derived antigens ex vivo have been proposed. Effective tumor immunity requires efficient loading of antigen and effective targeting of antigen-loaded DCs to reach secondary lymphoid organs. As this normally requires a maturation stimulus for the antigen-loaded DCs, various stimuli that serve as adjuvants for the anti-tumor response are under investigation. These include tumor-derived heat-shock proteins, pro-inflammatory cytokines, and direct activation of costimulatory molecules on
In some cases, antigen-loading and maturation stimuli are triggered by the same phagocytyically-competent receptor. This is the case for Fcγ receptors and complexes of tumor antigen and IgG. Ingestion of Dead Cells and Regulation of Inflammation; The Power of “Negative Signaling”

Apoptotic cell death is a consequence of cellular senescence or infection of cells with various microbial pathogens. Effective removal of these cells is required for tissue remodeling, for cross-priming, and for resolution of inflammation. This occurs by phagocytosis, which accomplishes the dual role of facilitating the clearance of apoptotic bodies and completing the cell death pathway. Many receptors on the phagocyte surface participate in this process; among these is the recently identified phosphatidylserine receptor (PSR), which recognizes PS exposed at the outer leaflet of the membrane of apoptotic cells and triggers the generation of TGF-β by the phagocyte. Recognition of apoptotic cells by phagocytes is enhanced by chemotactic stimuli released by apoptotic cells, such as lysophosphatidylcholine.

The consequences of ingestion of dead cells depends on the mechanism of cell death. Necrotic cells release a variety of pro-inflammatory substances, including heat shock proteins, which engage a subset of receptors on the phagocyte surfaces. These, in turn, bind cell surface proteins such as CD91, which potentially mediates endocytosis and cross-presentation. Heat shock proteins have been proposed to serve as adjuvants by binding toll-like receptors, although recent work has ascribed this function to contaminating LPS. In contrast, engagement of the PSR results in the production of TGF-β, which serves to dampen inflammation, especially mediated by macrophages. This may be critical in tuning the immune response; too much inflammation at an inappropriate time may result in excessive antigen presentation and, theoretically, autoimmunity (see below).

Another trigger of “negative signaling” is ligation of immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing receptors. In contrast to Fcγ receptors I, IIa, and III, FcγRIIb contains an ITIM in its cytosolic domain. Co-clustering of FcγRIIb with any other receptor results in an inhibition of phagocytosis and inflammation by recruitment of the SH2 domain-containing inositol 5’ phosphatase (SHIP). SHIP hydrolyzes the lipid product of PI 3-kinase, phosphatidylinositol-3,4,5-trisphosphate to phosphatidylinositol-3,4-bisphosphate, thereby limiting recruitment and activation of several PH domain-containing proteins, such as members of the Tec family of tyrosine kinases. These enzymes phosphorylate and activate phospholipase C-γ; therefore, recruitment of SHIP via ITIMs effectively inhibits signaling mediated by phospholipase C-γ, including calcium fluxes. Recruitment of SHIP via of FcγRIIb may be critical for the maintenance of tolerance as FcγRIIb−/− mice develop spontaneous autoimmunity in a B cell-autonomous fashion. Another type of ITIM bearing receptor, SIRPs, is expressed in myeloid cells and recruits the tyrosine phosphatase SHP-1 and SHP-2. One of its ligands, Surfactant Protein-A, is also an opsonin for apoptotic cells. Thus, apoptotic cells may generate mechanismically distinct inhibitory signals: production of TGF-β, a diffusible mediator, via the PSR, and recruitment of the cell autonomous inhibitor, SHP-1/2, by engagement of SIRPs. In other scenarios, SIRPs may serve as an “anti-phagocytic” receptor. For example, the presence of its counter-receptor on red blood cells, CD47, contributes to their lack of recognition as a phagocytic target. It has been suggested that failure of cognate interactions between CD47 on red blood cells and SIRPs on phagocytic cells may contribute to autoimmune hemolytic anemia.

In summary, by recruitment of enzymes that acts to antagonize tyrosine kinase-mediated signaling (SHP-1/SHP-2) or PI 3-kinase-mediated signaling (SHIP), or by generation of TGF-β, the program of inflammation that accompanies phagocytosis can be curtailed or potentially reversed.
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Individuals with various complement deficiencies are at greatly increased risk for developing autoimmune diseases. Interestingly, these same complement components are recognized opsonins for apoptotic cells. It has been suggested that decreased clearance of apoptotic bodies leads to an abundant source of potential autoantigens and predisposes to autoimmunity. This model is supported by the observation of anti-DNA antibodies in mice deficient in opsonizing complement components. In the case of Clq-deficient mice, this was accompanied by enhanced accumulation of apoptotic bodies, suggesting that Clq deficiency causes autoimmunity by impairment of the clearance of apoptotic cells. A similar correlation between delayed clearance of apoptotic cells and autoimmunity was observed in mice that lacked functional c-Mer, a tyrosine kinase that engages apoptotic cells and mediates their phagocytosis. Thus, the development of autoimmunity is associated with a defect in the phagocytic clearance of apoptotic cells. Despite the appeal of this interpretation, it is not known whether autoimmunity and defective phagocytosis are causally related. An alternative, though not mutually exclusive explanation, is that complement is required for presentation of self-antigen, such as chromatin, by complement receptor-bearing stromal cells in the bone marrow. These are presented to potentially immature autoreactive B cells leading to tolerization through clonal deletion and/or anergy. It is also possible that lack of appropriate phagocytic clearance of apoptotic cells results in an imbalance of pro- and anti-inflammatory signals, favoring the former. While this seems counterintuitive, as the “anti-inflammatory” PSR pathway of apoptotic cell clearance would be preserved under these circumstances, other anti-inflammatory pathways of corpse clearance may exist. For example, the acute phase reactant C-reactive protein binds to apoptotic cells and serves as both an opsonin and an inducer of TGF-β in a Clq-dependent fashion.

Conclusions

The study of phagocytosis and its assigned role in immunity has come a long way since Metchnikoff. Phagocytosis is now viewed as a fundamental mechanism of antigen presentation, both through MHC Class II and Class I (via cross-priming). This has implications for immunity not only to bacteria and fungi, but also to viruses and malignant cells. The dichotomy of “pro-inflammatory” and “anti-inflammatory” phagocytosis is useful in explaining how the immune system regulates the temporal response to infection. The balance between these pathways may prove to be a key determinant of the development of autoimmunity.

Acknowledgements

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References

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