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Commentary

Phagocytosis, Innate Immunity, and Host–Pathogen Specificity

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In mammals, phagocytosis is essential for a variety of biological events, including tissue remodeling and the continuous clearance of dying cells. Furthermore, phagocytosis represents an early and crucial event in triggering host defenses against invading pathogens, which is the focus of this commentary. Phagocytosis comprises a series of events, starting with the binding and recognition of particles by cell surface receptors, followed by the formation of actin-rich membrane extensions around the particle. Fusion of the membrane extensions results in phagosome formation, which precedes phagosome maturation into a phagolysosome. Pathogens inside the phagolysosome are destroyed by lowered pH, hydrolysis, and radical attack. As a result of this process, pathogen-derived molecules can be presented at the cell surface (antigen presentation), allowing the induction of acquired immunity (1). These early events that are mediated by the innate immune system are critical for host survival. Here we discuss new insights into how broad classes of microbial substructures are recognized by molecules of the innate immune system that are shared by many species, and the interspecies receptor diversity that has evolved to match distinct species-specific environmental challenges.

CEACAM3: A Phagocytic Receptor in Man.   Human beings are the only reservoir for *Neisseria meningitidis* and *N. gonorrhoeae*, *Haemophilus influenza*, and *Moraxella catarrhalis*. In this issue, Schmitter et al. (2) describe the elegant manner by which the human immune system recognizes these highly adapted species of commensal Gram-negative bacteria via a unique surface receptor known as carcinoembryonic antigen–related cell adhesion molecule 3 (CEACAM3, also known as CD66d). CEACAM3, a member of the CD66 family of receptors, which is now the subject of intense scrutiny, not only binds these uniquely human bacteria but mediates bacterial internalization (3, 4) via a signal transduction pathway that involves the small GTPase, Rac. CEACAM3 is a single chain molecule that is expressed exclusively on granulocytes and appears to function specif-

ically in the phagocytosis of commensal bacteria and the associated signal transduction events driven by these bacteria. Unlike the Toll receptor family of innate immune receptors, which has ancient orthologs in flies and other primitive organisms, CEACAM3 has no homologue in rodents and nonhuman primates, matching the species-specificity of *N. gonorrhoeae*, *H. influenza*, and *M. catarrhalis*. In the case of *N. gonorrhoeae*, CEACAM3 interacts with the 11-membered opacity (OPA) protein family. Accordingly, Schmitter et al. suggest CEACAM3 to be a phagocytic receptor for the bacterial surface proteins P5 of *H. influenzae* and UspA1 of *M. catarrhalis* (2), which are known to bind CEACAMs. OPA proteins, P5 and UspA1, undergo molecular variation over time, a mechanism that might help pathogens to evade the human immune response. What remains unanswered is how these proteins, which do not share overt homology, interact with the same receptor.

General Paradigms in the Phagocytosis of Microbial Pathogens. The findings on CEACAM3 and *Neisseria spp.* bring to mind a related broader question about innate immunity. How is the specificity of host–pathogen interaction achieved? In contrast to the highly species-specific interactions of CEACAM3 with commensals, the initial uptake of most microbes by phagocytes is typically comparatively nonspecific. First, the infectious particles become bound by serum components such as antibodies or complement components, a process known as opsonization. This allows the phagocyte to recognize the particle indirectly via Fc receptors (FcRs) and complement receptors (CRs). As outlined below, other surface receptors present at the time of the initial particle–phagocyte interaction can concurrently sample the particle during the phagocytic process. This enables the phagocyte to elicit a specific response to particles that determines the fate of both the invading organism and the host. Surprisingly, even the comparison of FcR- and CR-mediated particle uptake reveals remarkable differences in outcome: whereas FcR mediated phagocytosis is strongly proinflammatory, CR-mediated phagocytosis itself is noninflammatory (1, 5), even though some CRs may have coreceptor capabilities in mediating LPS responses (6).

The invasion of small numbers of bacteria can be assumed to be a frequent event, particularly for the species of *Neisseria*, *H. influenza*, and *M. catarrhalis* that commonly colonize the mucosal surfaces of humans. The immediate and nonphlo-

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gistic removal of bacteria from the blood and tissue prevents the host from succumbing to bacterial spread, septic mestastasis, and subsequent generalized inflammation and is a critically important feature of the innate immune system. Phagocytic leukocytes, including both tissue macrophages and granulocytes, play a crucial role in this process. Corresponding to the importance of early recognition of bacteria and the delivery of a cellular response, numerous proteins assemble at the point where the phagocyte membrane and the invading microbe meet. Extensive horizontal communication among the membrane proteins that multimerize at the developing phagosome and the delivery of multiple, overlapping and synergistic signals across the membrane are the consequences.

Evolutionary Conserved Particle Recognition and Initiation of Inflammation. In contrast to the CEACAMs, phagocyte receptors that trigger the transcription of inflammatory genes upon internalization of a microbe are remarkably conserved through evolution. The most important receptors for the initiation of inflammatory signals are the Toll-like receptors (TLRs) and proteins containing a nucleotide-binding oligomerization domain (NOD). The deficiency of specific TLRs and various adaptor proteins of the TLR signal transduction pathway, such as MyD88 and IL-1 receptor–associated kinase 4, have been shown to result in reduced bacterial clearance and a poor host responses to microorganisms, both in human beings and experimentally infected rodents (7, 8). The interaction between microbial particles and TLR pathways in granulocytes alone is highly complex, as exemplified for *Neisseria spp.* Among the multiple known molecular substructures of *Neisseria* that serve as ligands for phagocyte receptors, the lipopolysaccharide from *Neisseria* interacts exclusively with TLR4. TLR2, together with TLR1 and TLR6, recognize at least three cell wall components of *Neisseria*: peptidoglycan fragments, porins, and the Lip lipoprotein (9, 10). It has been reported that subtle changes in the degree of acylation of bacterial lipoproteins determines whether TLR1 or TLR6 serves as a coreceptor for TLR2, exemplifying the finely tuned nature of TLR recognition (11). Peptidoglycan fragments from *N. meningitidis* are also recognized by an intracellular receptor, NOD2, which drives transcriptional activation via NF-κB (12). Peptidoglycan fragments (MurNAc-L-Ala-D-IsoGln) from Gram-positive and –negative bacteria are detected by the intracellular receptor NOD2, whereas other peptidoglycan fragments (GlcNAc-MurNAc-l-Ala-γ-D-Glu-meso-DAP) specific for Gram-negative bacteria are detected by NOD1 (12). Thus NOD1 and NOD2 are striking examples of sophisticated solutions that allow the discrimination between different pathogens that manage to invade the intracellular space of cells.

Peptidoglycan Recognition Protein LC: A Phagocytic Receptor in Flies. As the role of the CEACAM, TLR, and NOD proteins becomes increasingly well delineated, alternative means of bacterial recognition are being identified also. As with the TLRs, an evolutionary perspective has helped us understand the potential role of these new proteins in host responses to bacteria. Immune-responsive cells in *Drosoph-*

ila melanogaster express a transmembrane cell surface receptor designated peptidoglycan recognition protein LC (PGRP-LC), which specifically recognizes Gram-negative bacteria. Recognition of Gram-negative bacteria by PGRP-LC activates a signaling pathway, which drives the expression of antibacterial peptide genes (13). The study of the 13 PGRPs in flies and their mammalian orthologs has led to some major surprises. For example, the discovery of TLR4 as the LPS receptor was directly due to work that others had done in *Drosophila.* Subsequently, other human TLRs were identified as peptidoglycan recognition proteins. Incredibly, fly Toll receptors (of which there are nine) apparently play no role in LPS or peptidoglycan recognition (14). Although four PGRP homologues exist in humans, they do not appear to be expressed as receptors by human macrophages (15). One mammalian PGRP, PGRP-L, is an enzyme that digests peptidoglycan, an *N*-acetylmuramyl-ʟ-alanine amidase. In fact, all PGRP proteins are structurally related to this family of enzymes, but only a subset are likely to be enzymatic. Mouse PGRP-S is not enzymatic but is somehow required for efficient neutrophil-based killing of nonpathogenic Gram-positive bacteria (16). The function of the two other mammalian PGRPs is unknown. PGRP-L may orchestrate highly specific cellular responses to Gram-negative bacteria such as *Neisseria*, but its role in immunity is still largely unknown. For all of our reliance on lower species of animals to inform us about the shape of the mammalian innate immune system, we are gradually coming back to the obvious conclusion that man is not a fly.

Rho Down the Stream. The interface between microbial particles and the phagocyte is critical for the specificity of the host response. Receptor–ligand interactions are at the heart of understanding how the innate immune system destroys microbes. However, downstream events are also important to both transcriptional activation of inflammatory genes and activity of the actin skeleton. In the case of TLRs, four intracellular proteins (MyD88, TRIF, MAL/TIRAP, TIRP/TRAM) are known to function as adaptors that connect the activation of TLRs to the transcription apparatus (17). In addition to these adapters, phosphatidylinositol-3′ kinase (PI-3K) has been found to associate with TLR2, a receptor for cell wall material of bacteria and parasites, via two putative binding domains (YxxM, YxxW) for the p85 subunit of PI-3K in the cytoplasmic domain of TLR2 (tyrosine residues 616 and 761). In turn, Rac1, a member of the Rho family of proteins, binds to PI-3K. The interaction between TLR2 and Rac1 appears to be important for transcriptional activation (NF-κB) in response to cell wall preparations from *S. aureus* (18). Interestingly, CEACAM3 contains similar YxxM motifs in the ITAM-like sequence of its cytoplasmic tail and colocalizes with Rac. Hence, it appears that *Neisseria* activate Rho proteins in a dual fashion via both TLR2 and CEACAM3.

Mounting evidence indicates that the Rho family of small GTPases, which consists of at least 16 mammalian members, mediates fine particle recognition by phagocytes. Rho proteins are often described as molecular switches be-

cause they cycle between an active (GTP-bound) to an inactive (GDP-bound) conformation similar to many hundred other GTPase switches in mammalian cells. The careful regulation of the GTPase activity is assured by over 60 activators and a similar number of inactivators (19). In addition, Rho proteins couple changes of the environment to intracellular signal transduction events. Greater than 60 targets of Rho activity have been identified to date (20). First, most Rho proteins affect the organization of polymerized actin (F-actin). RhoA, for example, induces actomyosin-based contractility leading to the formation of stress fibers. Rac and Cdc42 stimulate the formation of membrane extensions designated lamellipodia (Rac) and the more finger-like filopodia (Cdc42) (21). Furthermore, Cdc42 is essential for daughter cell growth of budding yeast. Rho proteins drive actin-based vesicle transport around the cell (22) and are involved in cell contraction and membrane blebbing, which are hallmarks of apoptotic cell morphology (23). Whereas effects on actin polymerization are probably the best studied functions of the Rho families, many other features of these proteins such as microtubule dynamics, adhesion, cell cycle progression, and control of gene transcription have been revealed.

It is exciting to ponder the observation that bacteria have evolved toxins that target the Rho family in order to escape the immune response. Clostridium species and *Staphylococcus aureus* secrete toxins that inactivate Rho proteins via ADP-ribosylation or glucosylation (24), whereas *Pseudomonas aeruginosa* and *Yersinia spp.* inject proteins similar to the GTPase-activating protein into eukaryotic cells via a type III secretion system. Seemingly paradoxical, highly sophisticated effects are elicited by the cytotoxic necrotizing factor 1 (CNF1) from *Escherichia coli*. CNF1 first activates members of the Rho family by deamidation of glutamine 63. This constitutive activation facilitates the entry of bacteria into nonprofessional phagocytes. Once bacterial uptake has been accomplished, however, CNF1 terminates the Rho activation by proteolytic degradation of Rho proteins via the 26S proteasome (25). It is tempting to speculate that this secondary down modulation of Rho activity represents an immune evasion mechanism of *E. coli*.

In conclusion, the innate immune system has multiple levels of specificity, some of which are shared, via evolutionary forces, with primitive organisms, and some of which are apparently unique to man. Upon contact of a phagocyte to a foreign particle, numerous surface molecules communicate details of the particle structure toward intracellular signaling pathways. Besides the molecules that have been mentioned here, many other surface proteins, such as the mannose receptors, DEC-205, dectin1, and scavenger receptors function as phagocytic receptors for invading microbes. The cumulative function of these and many more receptors of the human innate immune system appear to be specifically tailored to defend against systemic invasion of microorganisms that colonize mucosal and dermal surfaces. By comparing immunological solutions in phylogenetically very distinct species, new models of immune recognition can be established to better understand the pathogenesis of infectious diseases in man.

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