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Controlled One-on-One Encounters between Immune Cells and Microbes Reveal Mechanisms of Phagocytosis

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ABSTRACT Among many challenges facing the battle against infectious disease, one quandary stands out. On the one hand, it is often unclear how well animal models and cell lines mimic human immune behavior. On the other hand, many core methods of cell and molecular biology cannot be applied to human subjects. For example, the profound susceptibility of neutropenic patients to infection marks neutrophils (the most abundant white blood cells in humans) as vital immune defenders. Yet because these cells cannot be cultured or genetically manipulated, there are gaps in our understanding of the behavior of human neutrophils. Here, we discuss an alternative, interdisciplinary strategy to dissect fundamental mechanisms of immune-cell interactions with bacteria and fungi. We show how biophysical analyses of single-live-cell/single-target encounters are revealing universal principles of immune-cell phagocytosis, while also dispelling misconceptions about the minimum required mechanistic determinants of this process.

Many methods of the life and health sciences are designed to establish statistical confidence in a hypothesis, but they rarely provide definitive proofs. For example, most of our current insight into host-pathogen interactions has originated from cell and molecular bulk assays or from epidemiological studies, and is based on cumulative circumstantial evidence and correlative reasoning. The preferred subjects of many immunological studies are animal models or cell lines, even though it is often unclear how well insights from such studies carry over to the human immune system (1–3). The risks of translating such insights into medical applications cause growing concern for clinicians, patients, and entrepreneurs (4,5).

On the other hand, the rapid progress of gene sequencing is laying the groundwork for a much improved and potentially more personalized approach to medicine. Other recent key advances include the miniaturization of research tools and medical devices by micro- and nanobiotechnology. However, to fundamentally transform biomedicine, the development of gene catalogs and new technologies must be accompanied by conceptual innovation, in particular, an intensification of efforts to expose fundamental mechanisms (see Box 1) that govern biological behavior.

Tight control over one-on-one encounters between immune cells and microbes

Mechanistic analyses of single-live-cell encounters with microbes are scarce in the biomedical literature. This shortage can be attributed, among others, to methodological limitations of traditional single-cell experiments. For instance, it is inefficient to cosuspend immune cells and microbes in a microscope chamber and wait for chance encounters within the field of view. Moreover, adhesion of immune cells to a substrate tends to induce an activated state of the cells that differs dramatically from their quiescent state in suspension. For example, the production of reactive oxygen intermediates can vary as much as 100-fold between these two states under otherwise identical conditions (6).

Here, we discuss an alternative, interdisciplinary methodology that assesses fundamental mechanisms of phagocytosis by analyzing one-on-one encounters between immune cells and microbes (Figs. 1 and 2). These experiments use nonadherent cells, thus preventing premature cell activation. They offer superb control over cell-microbe contacts and have a time resolution of fractions of a second or better. They also facilitate an essentially axisymmetric configuration of the cell-microbe pair, which is viewed from the side, allowing us to visualize the interaction with great clarity (including, e.g., the onset of cell deformation, the closing of the phagocytic cup, or the changing cell-surface area), and to leverage experimental observations against computer simulations (7,8). As a consequence of these advantages, a spectrum of research questions can be addressed more directly than before, which enhances the strength of prospective evidence and often obviates the need for the accumulation of pieces of weaker evidence. The single-cell experiments have been validated with various types of microbes (9,10) (Fig. 1 *A*) and human immune (and other) cells (Fig. 1 *B*), and have already revealed insight into cellular behavior that had been inaccessible to traditional techniques. (For movies of representative single-live-cell experiments see https://www.youtube.com/user/HeinrichLab or the supplemental videos of (7,9–12)).

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BOX 1 What's in a mechanism?

The meaning of the term "mechanism" varies between different fields. Here, we follow the millennia-spanning tradition defining mechanisms as quantifiable relationships between causes and effects. In other words, a study is mechanistic (i.e., aimed at revealing mechanisms) if it addresses, in a quantitative manner, the lines connecting the dots. Knowledge about the exact nature of each dot is not essential to the understanding of a mechanism, although it is a useful bonus if available, as it defines particular instances of mechanisms and relates them to real-world questions. Studies that do not quantify causal relationships but only catalog ingredients of a process, such as discoveries of genes or gene products, or reports of the involvement of signaling molecules in pathway schemes, are examples of modern, molecular taxonomy. Taxonomy, of course, belongs to the realm of descriptive investigation, not mechanistic research.

It is important to note that this clarification is a matter of semantics, not judgment. Descriptive approaches are a vital part of the life sciences and have laid the groundwork for numerous discoveries. Optimal scientific progress can only be sustained if descriptive and mechanistic inquiries are in a healthy balance, and if this balance remains congruent with technological and conceptual advances.

The experiments have in common that quiescent immune cells are lifted above the chamber bottom before encountering a target, i.e., a bacterium, fungal particle, or surrogate model particle (Fig. 2). In principle, two methods can be used to accurately reposition microscopic objects in 3D: micropipetting and optical tweezers. Although it is possible to laser-trap individual cells (13), we found that optical tweezers are poorly suited to lift and hold immune cells for longer than a few seconds, because exposure to the focused laser beam tends to trigger visible changes in these highly excitable cells. Therefore, in all experiments discussed here the immune cell is held by gentle suction at the tip of a glass micropipette (Fig. 2). The microbe (or model particle) can be held either by optical tweezers (which is advantageous particularly for small objects like individual bacteria) or by a second micropipette (typically used to hold objects with diameters larger than ~1.5–2 μm and up to several tens of micrometers). With such a setup it is straightforward to bring a nonadherent immune cell into contact with a target particle and then examine the time course of the cell response.

Minimum set of mechanistic determinants of immune-cell phagocytosis

An ultimate goal of immune-cell-based host defenses is the neutralization of particulate pathogens by phagocytosis. Phagocytosis has been studied for more than a century. Most studies have investigated correlations between experimental conditions and the bulk efficiency of phagocytosis, assessed either biochemically (e.g., in terms of reactive oxygen species or cytokines), or as a count of target particles recognized by immune cells. These input-output correlations usually succeed in establishing whether or not a pathogen is protected from a given type of phagocyte, but it is rare that they reliably identify the mechanistic stage(s) at which the pathogen evades recognition. Hence, they may not provide the type of information that is essential for new rational (or bottom-up—as opposed to trial-and-error)

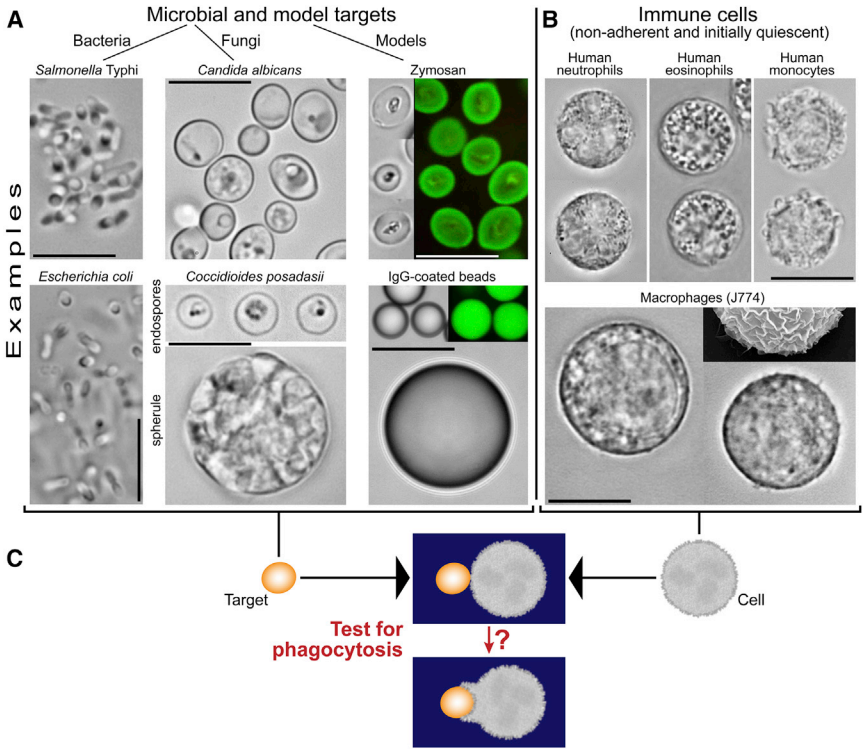

FIGURE 1 Single-live-cell approach to control one-on-one encounters between immune cells and microbes or surrogate particles. (*A*) Examples of microbes and model particles with a broad range of sizes. Strongly fluorescent zymosan particles (insoluble fraction from yeast cell walls) and microspheres are commercially available. (*B*) Examples of three types of human immune cells and a murine cell line. To enable meaningful comparative studies that start from a common baseline, meticulous attention is paid to keeping the cells in a nonadherent, initially quiescent state. The included SEM image shows the highly irregular microstructure of the surface of these cells. (*C*) To examine the mechanisms of phagocytosis, target particles are brought into contact with individual immune cells, and the time course of the cell response is analyzed. All scale bars denote 10 μm.

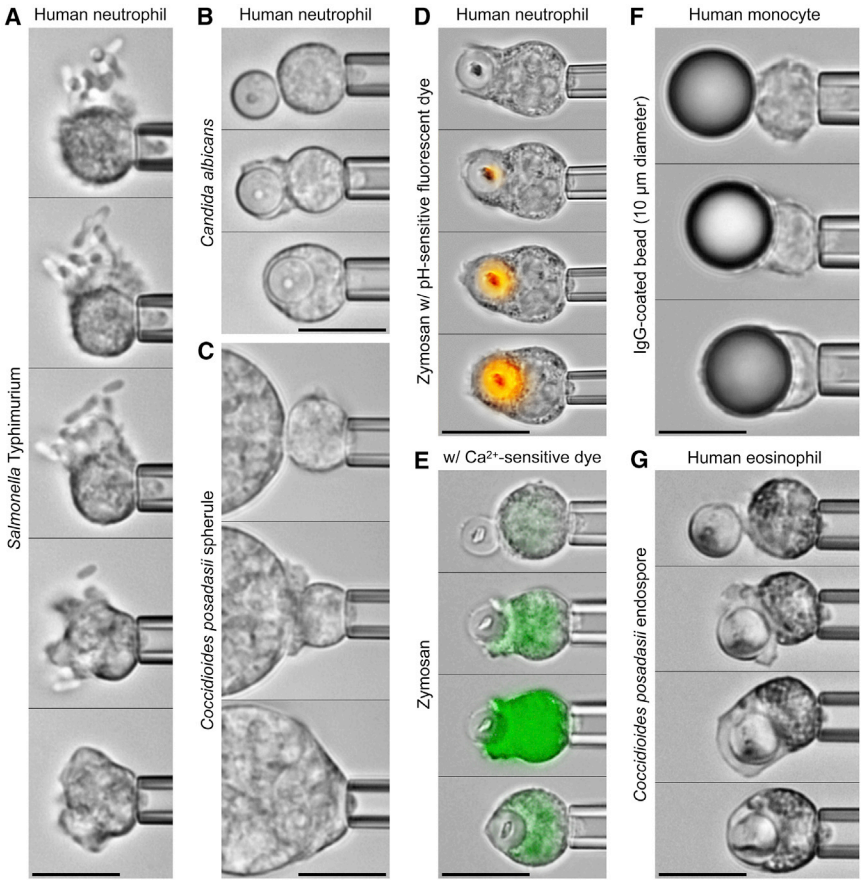

FIGURE 2 Single-live-cell phagocytosis of bacterial (*A*), fungal (*B*, *C*, and *G*), and model targets (*D–F*) by different types of human immune cells. The analysis of single-cell/single-target phagocytosis experiments (including frustrated phagocytosis of large targets, *C*) provides the timelines of cell morphology, cell-surface area, cortical tension, target trajectory, etc. Fluorescent imaging allows us to determine the timing of phagosome acidification (*D*) or of bursts of calcium signaling (*E*). Variations of experimental conditions reveal the roles of serum components and receptors, allowing us to characterize and rank the physiological relevance of different mechanistic routes of phagocytosis for each combination of cell and target type. All scale bars denote 10 μm.

approaches to the prevention, diagnosis, and treatment of infections.

An alternative strategy is to investigate the timeline of controlled one-on-one encounters between phagocytes and microbes in the absence of cell-substrate interactions (Fig. 2). A phagocyte typically takes no longer than a few minutes to engulf a particle that is not too large. During this time, the cell exhibits a remarkable surge of activity, including various signaling reactions, redistribution of signaling molecules and receptors, and remodeling of the cytoskeleton and cell membrane (14,15). Biophysical single-cell studies have begun to resolve, among others, the timelines of receptor engagement in relation to cell-surface topology (16), taking into account the nonuniform distribution of receptors between ridges and valleys of the cell surface (17). Other studies have examined the timeline of acidification of phagosomes after closure of the phagocytic cup (18) (see Fig. 2 *D*), the time course of intracellular calcium bursts triggered by cell contact with a target (19) (see Fig. 2 *E*), or the behavior of the cortical tension of immune cells during phagocytosis (20). Moreover, integrative single-cell experiments and computer simulations have illuminated how immune cells coordinate pushing and pulling actions to control processes such as the protrusion of the phagocytic cup, the expansion of the cell-surface area against the resistance of the rising cortical tension, the inward motion of the captured microbe, and the rounding of the cell at the end of engulfment (7,8).

Based on the quantitative analysis of such experiments, a set of universal mechanistic principles of immune-cell phagocytosis are emerging. Below we address six such principles in detail, hoping to lay the groundwork for an interdisciplinary framework that provides a useful context for more specialized cell and molecular phagocytosis studies. It is important to bear in mind that the discussed principles are not set in stone and may need to be revisited as new evidence emerges.

Cell-target adhesion is a critical element of phagocytosis

Immune-cell phagocytosis without adhesion is impossible. In rare cases, a newly forming endosome might by chance entrap a small particle without adhesion, but due to the lack of specificity and the small target size, this type of endocytosis does not usually qualify as immune-cell phagocytosis. Over the years, many studies have reported conditions that impair the engulfment of microbes. It is possible that in the majority of these cases, defective cell-target adhesion was the primary cause of the negative results.

Cell-target adhesion is mainly controlled by specific, weak bonds, i.e., noncovalent biomolecular interactions. Its overall strength is determined by the number of participating bonds and their individual strengths. Despite intense research on immune-cell receptors (15), detailed inquiries into the mechanistic functions of these receptors—including quantitative assessments of their numbers and dynamic binding strengths—remain scarce. For example, it is unclear to what extent specialized phagocytosis receptors support cell-target adhesion, or whether specialized adhesion receptors (which on their own would not initiate phagocytosis) are required for successful phagocytosis.

Previous studies implicate two immune-cell receptors as likely supporters of cell-target adhesion: complement receptor 3 (CR3), and Fcγ receptor IIIB (FcγRIIIB) (21–23). Both receptors interact mainly with opsonins coating the pathogen surface. CR3 (synonyms: Mac-1, CD18/CD11b) is an intriguing multifunctional hub that partakes in a variety of cell-motility processes. It is a β_2-integrin whose variable adhesive strength depends on its conformation. Among its multiple ligands are C3b (deposited onto the target surface by the complement system) as well as β-glucan (a constituent of fungal cell walls). CR3's role as an adhesion supporter of host-microbe interactions appears to be well established, but it is less clear whether CR3 acts exclusively as an adhesive receptor in this case.

In the absence of complement, innate immune cells still adhere readily to immunoglobulin G (IgG)-coated surfaces, which implies that Fcγ receptors can support cell-target adhesion as well. The two main IgG receptors of quiescent human neutrophils are FcγRIIA (CD32a) and FcγRIIIB (CD16b). Their low affinities suggest that individual bonds of these receptors cannot withstand appreciable pulling forces. However, passive human neutrophils display as many as ~3500 copies of FcγRIIIB per square micrometer of their surface (whereas the copy number of FcγRIIA is 10–20 times lower). FcγRIIIB is a GPI-linked receptor, lacking a cytoplasmic domain. A signaling role of FcγRIIIB has been reported (24,25), but the significance and mechanisms of the signaling biochemistry remain unclear. The high surface density of FcγRIIIB is a strong indicator that this receptor supports cell-target adhesion (as also affirmed by single-molecule studies (22)). In this case, the exceptionally large copy number of FcγRIIIB compensates for a low adhesive strength by facilitating the formation of multiple bonds with immobilized antibodies.

Active cytoskeletal remodeling is the main driver of outward (or forward) protrusion of deforming phagocytes

This active protrusive deformation (protrusive zipper, Fig. 3 *A*) is different from passive envelopment of particles by strongly adherent and essentially tension-free membranes (Brownian zipper, Fig. 3 *B*). The latter membranes can indeed spontaneously drape themselves around suitable objects, resulting in morphologies that resemble phagocytosis (Fig. 3 *B*). This alikeness has led to the proposition that strong adhesion between the phagocyte membrane and target surface might be the sole driving force of the engulfment of microbes (26), a notion that disregards forces produced by the actin cytoskeleton. However, a number of counterarguments dispel this misconception, including the following.

1) The literature on cell motility contains numerous reports of heightened F-actin density at the front of growing pseudopods, corroborating that actin polymerization lies at the core of a broadly conserved mechanism of protrusive cell deformation (15) (Fig. 3 *A*).
2) Disruption of the actin cytoskeleton with inhibitors (cytochalasin, latrunculin, etc.) effectively impairs immune-cell phagocytosis (12,27,28).
3) Partially protrusive phagocytosis morphologies that are incompatible with the notion of passive, adhesion-driven target envelopment can readily be observed (Fig. 3 *C*) and have been reported (12).
4) The energy balance of purely adhesion-driven particle envelopment is inconsistent with phagocytosis data. This balance offsets the favorable adhesion energy (parameterized by γ, the adhesion energy per unit area of cell-target contact) against the work required to expand the cell-surface area (overcoming the resistance of the cortical tension σ) (Fig. 3 *D*). Combined, these two energy contributions yield the Young-Dupré equilibrium condition that $\gamma \geq 2\sigma$ must hold for the completion of adhesion-driven envelopment of a particle (because the contact angle $\theta = 180^\circ$ when $\gamma = 2\sigma$; Fig. 3 *D*). The cortical tension of human neutrophils readily rises above 0.5 mN/m during the engulfment of large IgG-coated particles (20). For the full enclosure of such particles, the adhesion-energy density thus would have to exceed 10^{-15} J/μm^2. Using the conservative estimate of 10^{-6} M as an average binding constant of low-affinity Fcγ receptors (29), this adhesion energy would require ~20,000 exposed Fcγ receptors per square micrometer of neutrophil surface (and a similar density of Fc domains on the target surface). This number is >5 times the actual total copy number of (already very crowded) Fcγ receptors on the surface of human neutrophils, many of which are not available for cell-target adhesion because they are transiently ligated (due to the high concentration of 5–19 mg/mL of IgG in human serum (30,31)). Therefore, adhesion energy alone cannot drive the engulfment of particles by immune cells.
5) The previous argument (4) is based on a continuum model of adhesion. An alternative, more realistic view accounts for the discrete nature of adhesive receptor-ligand bonds. Considering these bonds as cross-bridges between two surfaces, it has been shown that, depending

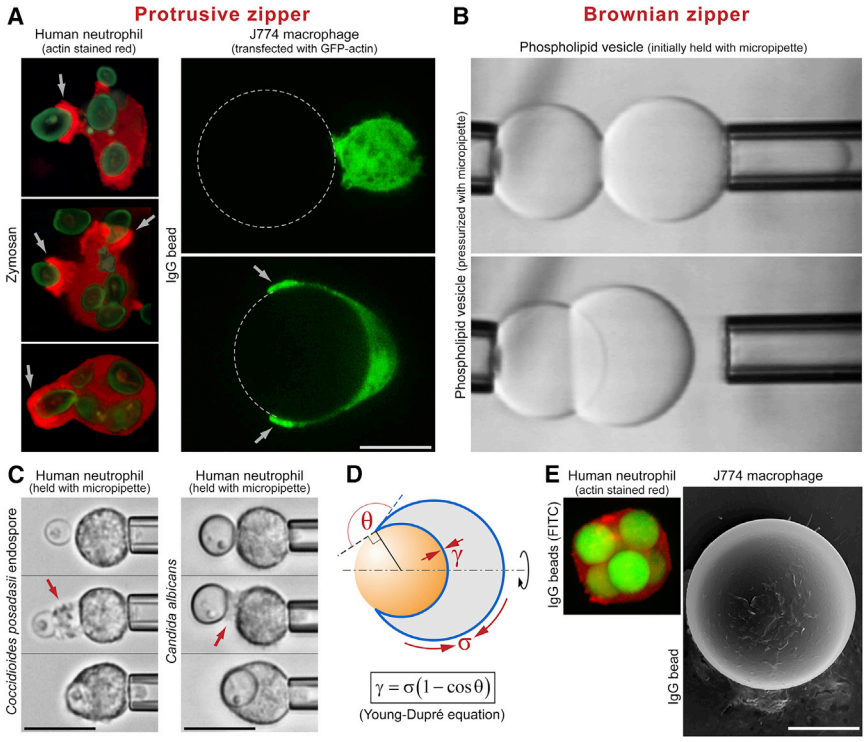

FIGURE 3 Assessment of universal mechanistic principles of immune-cell phagocytosis. (*A*) The high density of F-actin at the front of phagocytic cups (marked by *arrows*) affirms that active cytoskeletal protrusion is a key driving force of phagocytosis. (*B*) Classical demonstration of a Brownian zipper (adopted from (46)). Here, strong adhesion causes rapid partial envelopment of a pressurized lipid vesicle by another vesicle that possesses excess surface area. Although the resulting morphology resembles partial phagocytosis, the Brownian zipper model is inconsistent with mechanical analyses of immune-cell phagocytosis. (*C*) Partially protrusive neutrophil morphologies (as observed during the engulfment of fungal particles) are incompatible with the Brownian-zipper model, but have been successfully reproduced by computer simulations of the protrusive-zipper model (8). (*D*) The Young-Dupré equation predicts that in the case of a Brownian zipper, full engulfment of a spherical particle (where the contact angle $\theta = 180°$) requires an adhesion energy density γ that is twice the cortical tension σ. Measured values of σ show that the required adhesion energy would have to be unrealistically large to explain immune-cell phagocytosis on the basis of the Brownian zipper model. (*E*) A simple geometric analysis of cases where multiple or very large beads have been fully engulfed establishes that neutrophils and macrophages can expand their apparent surface area to a remarkable ~300% and ~500–600% of their resting areas, respectively (37). All scale bars denote 10 μm.

on their lateral spacing, "there is little or no tendency for the contact to spread unless the surfaces are forced together" (32). Thus, active protrusion at the front of the phagocytic cup is needed to enforce contact spreading between a phagocyte and its target.

A push-and-lock mechanism steers protrusion

As explained previously, the following cause-effect sequence governs the formation of most cellular protrusions. A local stimulus acting at the cell surface leads to reorganization of the adjacent cytoskeleton, which in turn creates a pushing force that displaces a membrane patch outward. This basic mechanism is consistent with the cell morphology observed during pure (i.e., cell-substrate-adhesion-free) chemotaxis (9–11,33). But it also implies that immediately after cell-target contact, a phagocyte should always form a protrusive pseudopod directly underneath the region of contact (where the phagocytosis-triggering stimulus is strongest). However, this prediction is at odds with the immediate formation of phagocytic cups at the onset of the engulfment of antibody-coated beads (11,12). The fronts of these cups advance along the particle surface rather than pushing the particle away. Hence, immune cells are able to steer a protruding pseudopod either toward a nearby target, or around an already attached particle.

Possible mechanistic routes that could account for this steering ability include (1) local suppression of actin polymerization (no push), (2) generation of a local, centripetal pulling force that counteracts a cytoskeletal outward push (push-and-pull), or (3) structural linkages that pin the engaged patch of target surface to the cytoskeleton of the phagocyte (push-and-lock). Experimental and computational evidence indicates that the third of these options dominates the steering behavior of immune cells (8,11,12). The actin cytoskeleton plays an intriguing dichotomous role in this process (12). It not only drives protrusion (push) but also participates in the local suppression of protrusion directed toward the affixed target (lock). For example, mild inhibition of actin decreases the normally large initial push-out distance of some fungal target particles (marked by *arrows* in Fig. 3 *C*), but increases this distance for antibody-coated beads (12).

Although the molecular details underlying this mechanism remain unclear, there are indeed many transmembrane proteins that are linked at least transiently to the actin cortex through cytoskeletal membrane anchors, such as talin (34), ERM (ezrin, radixin, moesin) proteins (35), and others. In the case of phagocytosis, serial linkages of the type target ligand ↔ transmembrane adhesion receptor ↔ cytoskeletal membrane anchor ↔ actin can pin the target surface to the cytoskeleton of the phagocyte. Through regulation of the overall strength of such connections, immune cells can

implement the decision whether to form pushing pseudopodial protrusions or enveloping pseudopodial cups (8,11).

Ample membrane reserves enable phagocytes to enclose microbes and grow in size

Plain lipid membranes can be stretched by at most 2–5% before they lyse (36). Phagocytes, on the other hand, can increase their surface area severalfold—human neutrophils to up to 300%, and J774 macrophages to up to 500–600% of their resting areas (37,38). This aptitude allows them to enclose multiple or large particles, and to accommodate the added volume of internalized microbes (Fig. 3 *E*). To achieve this enormous surface-area increase, phagocytes draw on preexisting membrane reservoirs, i.e., membrane wrinkles (scanning electron microscopy (SEM) image in Fig. 1 *B*) and internal vesicles (20,39,40). Wrinkles can exist in various forms, such as microvilli, ridges, pits, nanotubes, etc. They may be stabilized to varying degrees, contributing to the cell-surface area at different time points of phagocytosis. Fusion of vesicles with the plasma membrane not only increases the surface area, but also transports fresh membrane constituents (including receptors) to the cell surface. The content of fusing vesicles is inevitably released into the phagosome or environment, which can provide additional functionality such as cytokine secretion.

The cortical tension regulates the shape of phagocytes and pulls target particles inward

The cortical tension is a type of interfacial tension that opposes the expansion of the apparent surface area of phagocytes. In contrast to membranes of flaccid vesicles and red blood cells, the cortex of phagocytes is always under tension. Even quiescent phagocytes sustain a persistent resting tension that maintains their spherical shape (the shape of minimum surface area at given cell volume). To what extent the cortical tension rises during cell deformation depends on the type of phagocyte and on the severity of the deformation. For example, the tension of initially quiescent human neutrophils changes little during small deformations, but rises steeply when the surface area increases by ~30% or more (20). Moreover, the behavior of the tension depends on whether cell deformation is passive, i.e., imposed externally (for example, when a quiescent leukocyte is squeezed through narrow passages of blood vessels), or active, such as during phagocytosis. This dependence shows that phagocytes can actively regulate their cortical tension.

As a direct consequence of the predisposition of phagocytes to minimize their surface area, the cells round up after completion of target engulfment. But the tension plays an important role even earlier, by counterbalancing the protrusion of the phagocytic cup in a manner that effectively pulls the target particle inward. Single-cell experiments have established the following mechanism of target inward motion. Actin-driven protrusion creates fresh contact between the phagocytic cup and the microbial surface, and cell-target adhesion sustains the new contact. This adhesion is essentially irreversible, as observed in almost all experiments with neutrophils, monocytes, or macrophages, and in agreement with an analysis of cell adhesion mediated by discrete bonds (32). The cortical tension opposes the concurrent increase of the cell-surface area. Unable to relieve the resulting stress by reversing the adhesion-assisted protrusion, the phagocyte reduces its total surface area by widening the base of the phagocytic cup, gradually reincorporating the pseudopod into an overall rounding free part of the cell body. As a result, the distance between the centers of the cell and microbe decreases, causing the microbe to be pulled into the cell. This mechanism agrees well with the frequently observed synchronous onset of target inward movement and rise in cortical tension (10,12).

Remarkably, this mechanism does not require significant pulling action by molecular motors in a centripetal direction, as supported by computer simulations of phagocytosis (8). Instead, the main role of molecular motors during phagocytosis (in addition to membrane trafficking) appears to be the maintenance of the cortical tension (where the primary direction of motor action is parallel to the cell surface). Inhibition experiments have revealed that both actin and myosin are indeed required to sustain and regulate the tension.

The conserved ratio between cortical tension and cytoplasmic viscosity determines the rate of cell deformation

Whereas the cortical tension modulates cell deformation and causes phagocytes to round up, the effective cytoplasmic viscosity slows down changes of the cell shape (41,42). The ratio between these two quantities has units of velocity and sets the overall rate of deformation. Both the tension as well as the viscosity can vary significantly between different types of phagocyte; however, the tension/viscosity ratio appears to be broadly conserved, reflecting similar dynamics of target engulfment by different cell types (37). It makes sense that this ratio has a common optimum range for motile immune cells. Too high a viscosity would make the cell interior too rigid to allow any deformation within a reasonable time, and too high a tension would prevent the formation of local protrusions. On the other hand, if the cytoplasmic viscosity were too low, the cell body could not provide bracing support for developing protrusions. If the cortical tension were too low, phagocytes would be unable to round up, and the resulting irregular shapes would impede the cells' transport in the circulation. Interestingly, even though inhibition of actin or myosin alters both the cortical tension and the cytoplasmic viscosity, it affects the ratio between these two quantities to a lesser extent. This has been observed in actin-inhibition experiments

with passive cells (43,44), and it can also be inferred from the insignificant effect of mild actin or myosin inhibition on the speed of target inward motion during phagocytosis (even though all inhibitors lowered the tension) (12). Together, these observations suggest that the cortical tension and cytoplasmic viscosity are maintained by closely related molecular determinants.

Insignificant role of the bending resistance of the cell membrane

Notably absent from the previous principles of phagocytosis—although occasionally invoked in the literature (45)—is the membrane bending energy. Although the bending resistance of phagocyte membranes is expected to affect submicron (highly curved) morphological surface features of the cells, it is unlikely to play a significant role in the engulfment of micrometer-sized particles. For example, a human neutrophil performs work in excess of 10^{-14} J against the cortical tension when engulfing a particle of moderate size. In contrast, the typical energy cost due to bending of a lipid membrane during a comparable shape change is only of the order of 10^{-18} J, and is thus negligible on the relevant energy scale of immune-cell phagocytosis. This assessment is further supported by the ubiquitous presence of a myriad of high-curvature membrane wrinkles on the surface of phagocytes (SEM image in Fig. 1 *B*). Finally, even though it has become customary to treat cell membranes in terms of concepts and parameter values established for symmetric and isotropic lipid bilayers, the validity of this practice is questionable. Real biological membranes can potentially draw on a multitude of mechanisms to relieve bending stress (e.g., an asymmetric composition of the monolayers, enhanced flip-flop of lipids between them, anisotropy and nonuniform distribution of membrane constituents, domain formation, etc.), which could further reduce the energetic cost of membrane-curvature changes.

Outlook: from input-output correlation to mechanistic (bio)systems analysis

The previous discussion shows how a blend of single-live-cell methodology and cross-disciplinary reasoning has revealed new, to our knowledge, insight into the human immune response to microbial pathogens. Of importance, it is the integration of new tools and concepts that is leading to a better understanding of nature's nano- to microscale engineering principles—a prerequisite for future transformative breakthroughs in biology and medicine. At what depth single-cell research contributes to such understanding depends on whether it aims to describe input-output correlations or examines the actual system response, i.e., the sequence of cause-effect relationships that lead from input to output.

For example, several phagocytosis studies have reported variations in the behavior of immune cells as a function of the size, shape, or hardness of encountered target particles. These input-output studies often do not examine the cells' actual response program, and thus cannot discern whether a single, already known system response is merely producing input-dependent variations in cell behavior, or if the cells are switching between qualitatively distinct and potentially new system responses. Such studies usually cannot ascertain whether or not the cells are able to gauge a target's curvature or hardness. In contrast, a recent set of phagocytosis experiments expressly used particles of different sizes to inspect the basic response program of human neutrophils during encounters with antibody-coated targets (7). That study was able to predict the observed variations in phagocytic behavior in terms of a single cellular system response, implying that neutrophils do not possess a sensor of target curvature. The same approach also has revealed some of the essential mechanistic determinants of immune-cell phagocytosis discussed in this work.

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