Phosphoinositides and phagocytosis

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Phosphoinositide 3 kinases (PI3Ks)* are known as regulators of phagocytosis. Recent results demonstrate that class I and III PI3Ks act consecutively in phagosome formation and maturation, and that their respective products, phosphatidylinositol 3,4,5-trisphosphate (PI[3,4,5]P_3) and phosphatidylinositol 3-phosphate (PI[3]P), accumulate transiently at different stages. Phagosomes containing *Mycobacterium tuberculosis* do not acquire the PI(3)P-binding protein EEA1, which is required for phagosome maturation. This suggests a possible mechanism of how this microorganism evades degradation in phagolysosomes.

Phagocytosis is essential for the scavenging of dead cells and for eliminating invading microorganisms. It can occur by several mechanisms, of which the uptake of IgG-opsonized particles has been best characterized (Meresse et al., 1999). The maturation of the phagosome into a phagolysosome (Fig. 1) causes the degradation of phagocytosed particles by a combination of low pH, reactive oxygen metabolites and hydrolytic enzymes. Although it is an essential component of the innate immune defense, phagocytosis is also exploited by a number of intracellular pathogens, including the causative agent of tuberculosis, *Mycobacterium tuberculosis*, which thrives within phagosomes and infects a large proportion of the world's population (Russell, 2001). How do pathogens such as *M. tuberculosis* escape degradation in the phagolysosome? To answer this, we need to learn more about the molecules that govern phagosome maturation. Work presented in this (Vieira et al., 2001) and a previous issue (Fratti et al., 2001) indicates that PI3Ks, which phosphorylate phosphatidylinositol at the 3 position of the inositol headgroup (Fig. 2 A) (Vanhaesebroeck et al., 2001), are crucial players in phagosome formation and maturation.

The involvement of PI3Ks in phagocytosis was first indicated by the finding that PI3K inhibitors strongly inhibit phagocytosis of large particles (Araki et al., 1996). Because these compounds inhibit both class I and III PI3Ks, which produce PI(3,4,5)P_3 and PI(3)P, respectively, Vieira et al. (2001) have used more specific means to identify the roles of these two classes of PI3Ks in phagocytosis. By using cells in which the regulatory subunits of class I PI3Ks have been knocked out, they find that phagocytosis of large particles is strongly dependent on such PI3Ks. On the other hand, phagocytosis of smaller particles (<3 μm) can still proceed, suggesting that the requirement for type PI3Ks is correlated to the extent of actin reorganization, and the authors show evidence that phagosomes containing small IgG-coated latex beads can mature normally in the class I PI3K knockout cells. These results indicate that class I PI3Ks are required for phagocytosis of large particles, but not for phagosome maturation.

To study the involvement of the only known mammalian class III PI3K, VPS34, Vieira et al. (2001) and Fratti et al. (2001) microinjected phagocytes with an antibody inhibitory to this kinase. They show that phagocytosis is unaffected by the microinjected antibody, whereas phagosome maturation is inhibited. Thus, while class I PI3K, and by extension PI(3,4,5)P_3, is required for phagosome formation, class III PI3K, and by extension PI(3)P, is required for phagosome maturation.

The intracellular distribution of PI(3,4,5)P_3 and PI(3)P can be conveniently studied by transfecting cells with green fluorescent protein–tagged PH, PX, and FYVE domains that bind to these PI3K products with high affinity and specificity (Balla et al., 2000). Such studies have recently shown that PI(3,4,5)P_3 is formed at the phagosomal cup, and that it rapidly disappears after the phagosome has been sealed off from the plasma membrane (Marshall et al., 2001). The disappearance of PI(3,4,5)P_3 is most likely mediated by phosphatases that are recruited to the newly formed phagosome (Ellson et al., 2001a). Previously, it was thought that the cellular levels of PI(3)P are constant. However, Vieira et al. (2001) and Ellson et al. (2001b) now show that a high level of PI(3)P is formed in the phagosome membrane immediately after sealing from the plasma membrane and that the PI(3)P remains for several minutes. This demonstrates that there is a sequential formation and turnover of PI(3,4,5)P_3 and PI(3)P during phagosome formation and maturation, consistent with the respective roles of class I and III PI3Ks in these processes.

How do PI(3,4,5)P_3 and PI(3)P regulate phagocytosis and phagosome maturation? A likely role of PI(3,4,5)P_3 is to recruit proteins that control the actin cytoskeleton. Candidate proteins are Vav and ARNO, which bind to PI(3,4,5)P_3 and act as GTP/GDP exchange factors for small GTPases that are known to regulate actin remodelling. These include Arf6

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*Abbreviation used in this paper: PI3K, phosphoinositide 3 kinase.

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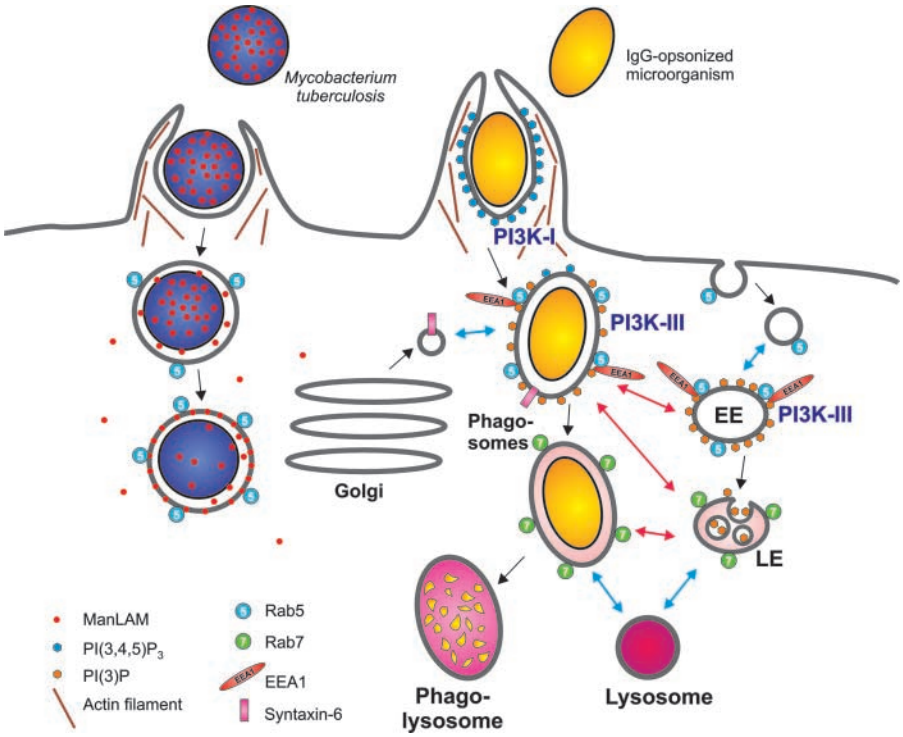

Figure 1. **Phagocytosis of *M. tuberculosis* and an IgG-opsonized microorganism.** The IgG-opsonized microorganism binds to Fcγ-receptors in the membrane of the phagocyte. This causes phosphorylation of the cytoplasmic part of the receptor and subsequent recruitment of a class I PI3K. The PI3K produces $PI(3,4,5)P_3$, which recruits proteins involved in the rearrangements of cortical actin that are necessary for membrane remodelling and phagosome formation. When the phagosome is sealed, $PI(3,4,5)P_3$ is rapidly dephosphorylated, and PI(3)P is formed by a class III PI3K, which is recruited to the early phagosome by the small GTPase Rab5 (Christoforidis et al., 1999). EEA1, a protein that promotes the tethering and fusion of early endocytic organelles, is also recruited by the interaction with PI(3)P and Rab5. Through exchange of molecules with cytosol, acquisition of cargo from the biosynthtic pathway and transient ("kiss-and-run") fusion (red double arrows) with early endosomes (EE) and late endosomes (LE), the phagosome gradually matures (Desjardins et al., 1994). Eventually it fuses (blue double arrow) with a lysosome, thus forming a phagolysosome in which degradation of the microorganism ensues. Note that early phagosomes and EEs contain Rab5, whereas more mature phagosomes and LEs contain Rab7. *M. tubercolusis* also enters the phagocyte by phagocytosis, but the phagosome does not mature, and it does not acquire EEA1. The shedding of ManLAM, which intercalates into phagosomal and other membranes, contributes to the maturation arrest, possibly by preventing EEA1 recruitment.

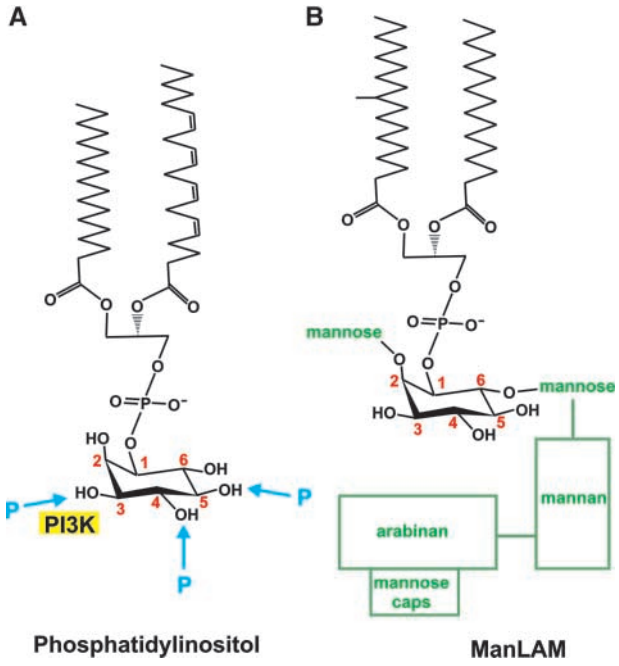

Figure 2. **The structures of phosphatidylinositol (A) and ManLAM (B), a glycophosphatidylinositol from *M. tuberculosis*.** Phosphatidylinositol can be phosphorylated in the 3, 4, or 5 positions of the inositol headgroup, as indicated with blue arrows. PI(3)P is phosphorylated at the 3 position, and $PI(3,4,5)P_3$ at the 3, 4, and 5 positions. Adapted from Vanhaesebroeck et al. (2001) and Gilleron et al. (2000).

(regulated by ARNO) and Rac1 (regulated by Vav), which have been directly implicated in phagocytosis (Vanhaesebroeck et al., 2001). As for the role of PI(3)P, Fratti et al. (2001) find that antibodies against EEA1, a PI(3)P-binding effector of the small GTPase Rab5 (Simonsen et al., 1998) which is present on early phagosomes and endosomes, inhibit phagosome maturation. This suggests that EEA1-mediated membrane tethering and fusion is critical for this process to occur. The mechanism by which EEA1 regulates phagosome maturation remains to be characterized, but it is worth noting that EEA1 interacts with syntaxin-6, a protein that is thought to mediate the fusion of a subset of Golgi complex–derived vesicles with early endosomes and phagosomes (Fig. 1) (Simonsen et al., 1999). Therefore, EEA1 could play a role in the phagosomal acquisition of certain cargo molecules from the biosynthetic pathway.

Even though the new studies provide significant insight into the role of phosphoinositides in phagosome maturation, several issues remain to be clarified. One concerns PI(3)P, whose generation does not always require a class III PI3K. Phosphoinositide phosphatases clearly play a role in the turnover of $PI(3,4,5)P_3$ and PI(3)P. In activated neutrophils, there is evidence that phosphoinositide 5- and 4-phosphatases convert $PI(3,4,5)P_3$ into PI(3)P, which activates the phagocyte oxidase complex (Ellson et al., 2001a). Moreover, class II PI3Ks, which are insensitive to standard PI3K inhibitors, can also produce PI(3)P (Vanhaesebroeck et al., 2001), and the transient accumulation of PI(3)P on *Salmonella*-containing phagosomes does not appear to depend on class I or III PI3Ks

(Pattni et al., 2001). Another issue concerns the possible role of PI(3)P-binding proteins other than EEA1 in phagosome maturation. The human genome encodes >40 PI(3)P-binding proteins, among which potential regulators of phagosome maturation may well exist.

Can our recent knowledge about the roles of PI(3,4,5)P_3 and PI(3)P in phagocytosis be exploited to fight tuberculosis? Fratti et al. (2001) find that mycobacterial phagosomes, even though they contain Rab5, fail to recruit EEA1 and syntaxin-6, and that phagosomes that contain latex beads coated with ManLAM (Fig. 2 B), a glycophosphatidylinositol that is shed by mycobacteria (Beatty et al., 2000), show inefficient EEA1 recruitment and maturation. Exactly how ManLAM inhibits EEA1 recruitment is not known yet, but one possibility is that it inhibits the formation of PI(3)P. Since PI(3)P is also required for activation of the phagocyte oxidase complex (Ellson et al., 2001a), inhibition of PI(3)P production might also protect *M. tuberculosis* from oxidative damage. Obviously, it will now be interesting to study the formation of PI3K products during phagocytosis of *M. tuberculosis*. These new findings suggest one mechanism by which *M. tuberculosis* may prevent phagosome maturation, and drugs that specifically target the biosynthesis of ManLAM might be interesting candidates for treating tuberculosis.

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