NK-cell function as a whole during the chronic phase of infection. Clearly, the study by Shah et al. (2010) marks only the beginning of a fascinating story that will shed new light on an important but still poorly understood aspect of the interaction of HIV-1 with NK cells.

REFERENCES

Alter, G., and Altfeld, M. (2006). Curr. Mol. Med. *6*, 621–629.

Bonaparte, M.I., and Barker, E. (2003). AIDS *17*, 487–494.

Bryceson, Y.T., March, M.E., Ljunggren, H.G., and Long, E.O. (2006). Immunol. Rev. *214*, 73–91.

Cohen, G.B., Gandhi, R.T., Davis, D.M., Mandelboim, O., Chen, B.K., Strominger, J.L., and Baltimore, D. (1999). Immunity *10*, 661–671.

Kirchhoff, F. (2010). Cell Host Microbe *8*, 55–67.

Lanier, L.L. (2008). Nat. Rev. Immunol. *8*, 259–268.

Moll, M., Andersson, S.K., Smed-Sorensen, A., and Sandberg, J.K. (2010). Blood *116*, 1876–1884.

Richard, J., Sindhu, S., Pham, T.N., Belzile, J.P., and Cohen, E.A. (2010). Blood *115*, 1354–1363.

Shah, A.H., Sowrirajan, B., Davis, Z.B., Ward, J.P., Campbell, E.M., Planelles, V., and Barker, E. (2010). Cell Host Microbe *8*, this issue, 397–409.

Ward, J., Davis, Z., DeHart, J., Zimmerman, E., Bosque, A., Brunetta, E., Mavilio, D., Planelles, V., and Barker, E. (2009). PLoS Pathog. *5*, e1000613. 10.1371/journal.ppat.1000613.

Caging Targets for Destruction

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Intracellular bacterial pathogens engage in a tug-of-war with innate host defenses. In this issue of *Cell Host & Microbe*, Mostowy et al. (2010) identify a role for the septin family of cytoskeletal proteins in targeting intracellular *Shigella* to the autophagy pathway.

Several pathogenic bacteria, including *Listeria monocytogenes*, *Salmonella* spp., *Legionella pneumophila*, *Yersinia* spp., and *Shigella* spp., cause disease by entering into and surviving within cells. These intracellular bacteria engage in a tug-of-war with host cell defenses, being pulled between bacterial restriction and death on one side and dissemination and survival on the other. In the time immediately after infection, the former is mediated to a large extent by the host cell autophagy pathway. In this pathway, intracellular bacteria are engulfed in intracytosolic membrane-bound vacuoles called autophagosomes that fuse with lysosomes, leading to bacterial destruction (Deretic and Levine, 2009). *Shigella* spp. are among a subset of intracellular bacterial pathogens that, after entry into cells, escape the vacuole and replicate in the cytosol. Once in the cytosol, each member of this group, which includes *Shigella* spp., *L. monocytogenes*, *Burkholderia* spp., *Rickettsia* spp., and *Mycobacterium marinum*, induces actin polymerization on its surface, which propels it to the cell periphery, whereupon it spreads into adjacent cells (Stevens et al., 2006). The ability of these organisms to spread directly into adjacent cells is critical to virulence. Of the population of organisms that enter the cytosol in a given infection, a substantial percentage escape autophagy, while others are brought into the autophagy pathway where they are destroyed (Ogawa et al., 2005). The intracellular fate of these cytosolic bacteria is a delicate balance between bacterial and host factors. For *Shigella*, autophagy proteins bind the bacterial protein IcsA (VirG), which is required for actin-based motility, and this interaction is inhibited by a second bacterial protein, IcsB (Ogawa et al., 2005). Few host factors that determine the fate of intracellular *Shigella* have been previously identified. In this issue, Mostowy et al. (2010) demonstrate a role for the host septin family of cytoskeletal proteins in targeting intracytosolic *Shigella flexneri* to the autophagy pathway.

Septins are a large class of proteins involved in many diverse cellular processes that have been shown to functionally interact with cellular membranes and the cytoskeleton. Originally discovered in 1971 through a genetic screen for yeast mutants defective in budding, septins assemble into rings at the yeast bud neck and are thought to act as membrane diffusion barriers that separate the mother from the daughter cell (Cao et al., 2009). Septins are conserved across animals and fungi, but are absent in plants. Humans have 14 septins that are divided into four groups, among which there is partial functional redundancy. Importantly, septins from the different groups form hetero-oligomeric protein complexes that assemble into nonpolar filaments (Weirich et al., 2008). The variation in septin proteins and their ability to form higher order hetero-oligomeric structures translates into many combinatorial possibilities, leading to distribution throughout the cell and multiple diverse protein interactions and functions.

Several notable functions of septins relate to their ability to interact with membranes. In addition to serving as

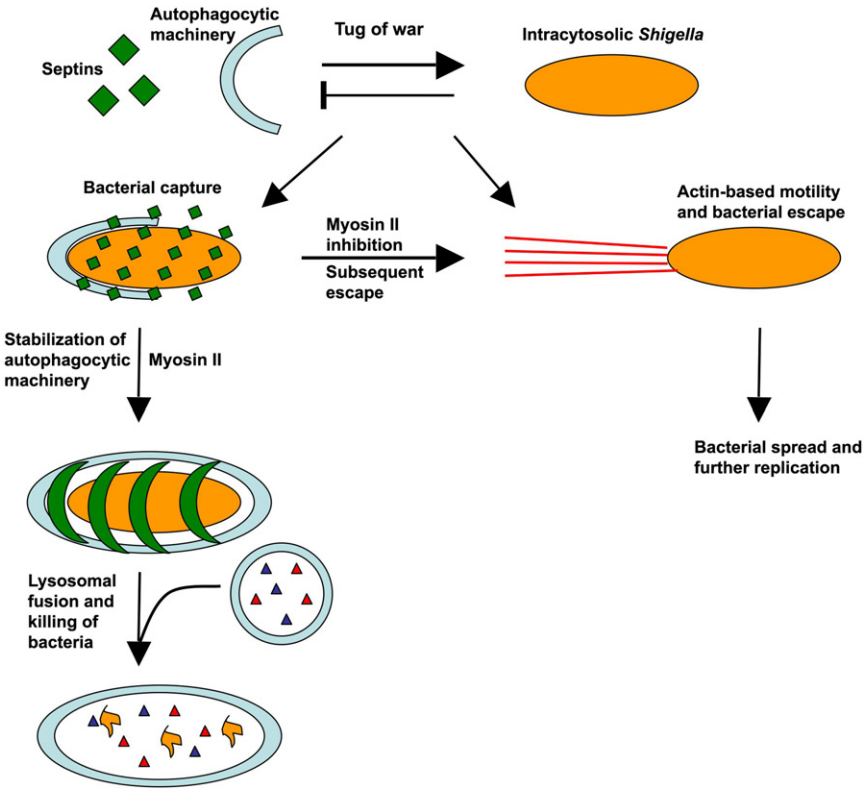

Figure 1. Model of the Tug-of-War between Intracytosolic *Shigella* and Host Cell Defenses Early after Cellular Infection, as Demonstrated by Mostowy et al. in the Current Issue of *Cell Host & Microbe*

A subset of intracytosolic *Shigella* polymerizes actin tails and spreads into adjacent cells, while other intracytosolic *Shigella* are trapped in cages formed by cellular septin proteins. Septin cages play a critical role in targeting *Shigella* to the autophagocytic pathway. Myosin II contributes to the targeting of *Shigella* to the autophagosome, and its inhibition enables *Shigella* to escape from septin cages.

membrane diffusion barriers in yeast, in mammalian cells, septins interact with the plasma membrane via a conserved polybasic region in the N terminus that binds membrane-associated phosphatidylinositol (4,5)-bisphosphate and phosphatidylinositol (3,4,5)-triphosphate (Cao et al., 2009). Moreover, the mammalian septin SEPT5 plays a role in the fusion of exocytic vesicles with the plasma membrane through its direct association with SNARE proteins (Cao et al., 2009). The intrinsic bent shape of septin filaments seen in crystal structures may allow for it to accommodate interactions with the curvature of membranes.

While able to connect with cellular membranes on one hand, septins interact with both the actin and the microtubule cytoskeleton on the other (Spiliotis, 2010). Biochemical reconstitution studies show that septins polymerize into filaments using F-actin fibers as a template and actin-binding proteins such as myosin II as adaptors. Six types of mammalian septins have been shown to colocalize with microtubules, in a process that may be facilitated by an association of microtubules with the GTPase domain at the N terminus of septins. The GTPase binding domain and the membrane-binding polybasic domain, which interacts with membranes, form distinct interfaces in the crystal structure (Weirich et al., 2008), suggesting that simultaneous interaction with membrane and cytoskeleton may be possible. Given their ability to interact with both membranes and the cytoskeleton, septins are ideally poised to function in cellular trafficking. Indeed, SEPT2 and SEPT11 localize with cortical actin at the plasma membrane during Fc receptor phagocytosis (Cao et al., 2009).

In the current issue of *Cell Host & Microbe*, Mostowy et al. (2010) make a compelling argument that septins play a critical role in barricading the intracellular trafficking of *Shigella*, thereby inhibiting dissemination and survival. Only a subset of intracytosolic *Shigella* assembles actin tails. Mostowy et al. demonstrate that intracytosolic *Shigella* without actin tails are surrounded by septin "cages"; using immunofluorescence, they show that the septins SEPT2, SEPT9, and SEPT11 each form spirals along the length of the bacterium. Interestingly, these three septins belong to different groups in the septin family and therefore, in theory, may be associating into a hetero-oligomeric complex. Using interference RNA to deplete SEPT2 or SEPT9, the authors demonstrate a functional role for each of these two septins, finding that in their absence, septin cages are formed significantly less frequently and the percentage of intracellular bacteria associated with actin tails is significantly increased. SEPT11 depletion does not inhibit colocalization of SEPT2 and SEPT9, perhaps due to functional redundancy that does not apply to SEPT2 and SEPT9. The association of septin cages predominantly with *Shigella* that lack actin tails is consistent with a model in which intracytosolic *Shigella* are either encaged by septins in conjunction with other cellular factors or are able to escape host cell capture by assembly of an actin tail (Figure 1).

Consistent with prior publications of an association of septins with cytoskeletal components (Cao et al., 2009; Spiliotis, 2010), Mostowy et al. (2010) show that host cytoskeletal actin, N-WASP, and mysosin II not only colocalize with but also have functional roles in the formation of septin cages around *Shigella*. The presence of actin polymerization and N-WASP are required for the association of septins with *Shigella* (Mostowy et al., 2010), perhaps contributing to the previously reported requirement for IcsA in the recruitment of autophagy markers (Ogawa et al., 2005). Labeled actin or N-WASP forms spirals that alternate with those of septins, suggesting that each one's role in septin assembly is indirect. In contrast, myosin II labeling overlaps with septin, perhaps indicating that myosin II plays a more direct role in septin assembly or stabilization. The results are consistent with the ability of septins to use F-actin as a template for filament formation with myosin II functioning as an adaptor between the two. Using

pharmacologic inhibitors, Mostowy et al. (2010) show that association of septin cages around *Shigella* is dependent upon myosin II activity. Disruption of myosin II activity enables bacteria to "break free" from the septin cage and initiate actin-based motility, clearly demonstrating the switch from one intracellular fate, that of septin association, to another, that of actin polymerization and intercellular spread (Figure 1).

Most interesting among the findings from Mostowy et al. (2010) is a previously unrecognized link between septins and the autophagy pathway in mammalian cells. Many markers of the autophagy pathway colocalize with *Shigella* in septin cages, suggesting that the purpose of the cages may be not only to prevent actin polymerization, tail formation, and spread of bacteria, but also to target intracytosolic bacteria for degradation. Mutant *Shigella* lacking IcsB have been shown to enter the autophagy pathway with increased frequency (Ogawa et al., 2005). Mostowy et al. (2010) found that an *icsB* mutant is significantly more frequently associated with septin cages, while an *icsA* mutant, which has been previously shown to be relatively resistant to autophagy (Ogawa et al., 2005), is significantly less frequently associated with cages. These results seem to suggest a codependence of septins with factors that mediate autophagy; when septins are absent, autophagy markers accumulate less, and vice versa. Thus, these results indicate an ongoing pull between host and bacterial factors in determining the fate of intracellular *Shigella*.

The dynamics of higher order septin assembly and function is complex and incompletely understood, yet is likely coordinated by a variety of intracellular and extracellular cues. The findings presented in Mostowy et al. (2010) suggest that one player in this process is the proinflammatory cytokine TNF-α, which is known to be important in host responses to intracellular bacteria. Addition of TNF-α to cells prior to infection with *Shigella* increases septin assembly on intracytosolic bacteria and correlates with less frequent actin tail formation and bacterial dissemination. Thus, the cytokine TNF-α modulates septin assembly and targeting of cytosolic *Shigella* to the autophagy pathway.

Taken together, the findings presented in Mostowy et al. (2010) demonstrate a continuous struggle between intracytosolic *Shigella* and the host autophagy pathway, in which the assembly of septin cages plays a critical role (Figure 1). Precisely how these findings can or will be generalized to other intracellular bacteria is uncertain. The authors indicate that while *L. monocytogenes* and *Rickettsia conorii* do not associate with septin cages, *M. marinum*, which is thought to have a mechanism of actin polymerization more similar to that of *Shigella*, does associate with them. These data suggest that certain mechanisms of actin polymerization may be better suited for septin-mediated entrapment than others. Whether *L. monocytogenes* and *R. conorii* might be captured by a parallel septin-independent pathway and whether other intracellular bacterial pathogens are captured by a septin-dependent mechanism remain unknown. Nevertheless, this paper contributes to our insight into the mechanisms involved in the tug-of-war between host and intracellular pathogen, identifying septin proteins as key players in this struggle.

REFERENCES

Cao, L., Yu, W., Wu, Y., and Yu, L. (2009). Cell. Mol. Life Sci. *66*, 3309–3323.

Deretic, V., and Levine, B. (2009). Cell Host Microbe *5*, 527–549.

Mostowy, S., Bonazzi, M., Hamon, M.A., Tham, T.N., Mallet, A., Lelek, M., Gouin, E., Demangel, C., Brosch, R., Zimmer, C., et al. (2010). Cell Host Microbe *8*, this issue, 433–444.

Ogawa, M., Yoshimori, T., Suzuki, T., Sagara, H., Mizushima, N., and Sasakawa, C. (2005). Science *307*, 727–731.

Spiliotis, E.T. (2010). Cytoskeleton (Hoboken) *67*, 339–345.

Stevens, J.M., Galyov, E.E., and Stevens, M.P. (2006). Nat. Rev. Microbiol. *4*, 91–101.

Weirich, C.S., Erzberger, J.P., and Barral, Y. (2008). Nat. Rev. Mol. Cell Biol. *9*, 478–489.