Virtually all of the available hydrogen in the intestine originates from microbial fermentation of carbohydrates. In this study, analysis of all publicly available metagenomes confirmed that hydrogen production represents a universal feature of the cecal microbiota in the intestinal tract of animals and humans, including LCM mice. In contrast, in both germ-free mice and mice treated with antibiotics, hydrogen was absent or low, and consequently a functional Hyb hydrogenase did not provide a colonization advantage to *Salmonella*. Moreover, as some intestinal microbes consume hydrogen, converting it to methane or hydrogen sulfide, the amount of intestinal hydrogen is directly influenced by the microbiota's balance between "hydrogen producers" and "hydrogen consumers." Consistent with this idea, precolonization of LCM mice with hydrogen consumers reduced the availability of hydrogen and thusly hampered Hyb-dependent colonization by *Salmonella*.

Altogether, this study sheds light on the complex interaction between *Salmonella* and the microbiota. On the one hand, the microbiota is known to provide colonization resistance to *Salmonella* infection, as antibiotic treatment increases the susceptibility to *Salmonella* both in experimental and in clinical settings. On the other hand, the microbiota is exploited by *Salmonella*, which utilizes the microbiota-derived hydrogen to replicate to higher levels. As the availability of nutrients can affect the composition of the gut microbiome, and thus potentially hydrogen production, this work also suggests that infection risk may depend in part on dietary habits and in part on the microbial balance between hydrogen producers and hydrogen consumers. Specifically, colonization with hydrogen consumers may protect against the initial ecosystem invasion by *Salmonella* and likely by other pathogens that may utilize Hyb hydrogenases for their initial replication. In contrast, at later stages of infection, hydrogen consumers that release hydrogen sulfide may enhance the growth of *Salmonella* by cooperating with the host to form tetrathionate (Winter et al., 2010). Nevertheless, manipulation and modulation of the microbiota and its metabolic functions may provide potential targets to reduce the colonization and expansion of *Salmonella* and other enteric pathogens.

## REFERENCES

Lawley, T.D., and Walker, A.W. (2013). Immunology *138*, 1–11.

Liu, J.Z., Jellbauer, S., Poe, A.J., Ton, V., Pesciaroli, M., Kehl-Fie, T.E., Restrepo, N.A., Hosking, M.P., Edwards, R.A., Battistoni, A., et al. (2012). Cell Host Microbe *11*, 227–239.

Lopez, C.A., Winter, S.E., Rivera-Chavez, F., Xavier, M.N., Poon, V., Nuccio, S.P., Tsolis, R.M., and Bäumler, A.J. (2012). MBio. *3*, e00143-12.

Maier, L., Vyas, R., Cordova, C.D., Lindsay, H., Schmidt, T.S.B., Brugiroux, S., Periaswamy, B., Bauer, R., Sturm, A., Schreiber, F., et al. (2013). Cell Host Microbe *14*, this issue, 641–651.

Raffatellu, M., George, M.D., Akiyama, Y., Hornsby, M.J., Nuccio, S.P., Paixao, T.A., Butler, B.P., Chu, H., Santos, R.L., Berger, T., et al. (2009). Cell Host Microbe *5*, 476–486.

Stecher, B., Barthel, M., Schlumberger, M.C., Haberli, L., Rabsch, W., Kremer, M., and Hardt, W.D. (2008). Cell. Microbiol. *10*, 1166–1180.

Thiennimitr, P., Winter, S.E., and Bäumler, A.J. (2011a). Curr. Opin. Microbiol. *15*, 108–114.

Thiennimitr, P., Winter, S.E., Winter, M.G., Xavier, M.N., Tolstikov, V., Huseby, D.L., Sterzenbach, T., Tsolis, R.M., Roth, J.R., and Bäumler, A.J. (2011b). Proc. Natl. Acad. Sci. USA *108*, 17480–17485.

Winter, S.E., Thiennimitr, P., Winter, M.G., Butler, B.P., Huseby, D.L., Crawford, R.W., Russell, J.M., Bevins, C.L., Adams, L.G., Tsolis, R.M., et al. (2010). Nature *467*, 426–429.

Zelante, T., Iannitti, R.G., Cunha, C., De Luca, A., Giovannini, G., Pieraccini, G., Zecchi, R., D'Angelo, C., Massi-Benedetti, C., Fallarino, F., et al. (2013). Immunity *39*, 372–385.

# *Streptococcus pyogenes* Escapes from Autophagy

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**Autophagy is important for innate defense against intracellular bacteria, such as Group A *Streptococcus* (GAS). In this issue of *Cell Host & Microbe*, Barnett et al. (2013) demonstrate that the globally disseminated serotype M1T1 clone of GAS can evade autophagy via streptococcal cysteine protease SpeB-mediated degradation of ubiquitin-LC3 adaptor proteins.**

Group A *Streptococcus* (GAS or *Streptococcus pyogenes*) is a strictly human pathogen that normally colonizes the throat and skin without causing disease. Members of this species are classified into over 100 serotypes by the immunogenic differences in their surface M protein and polymorphisms in their *emm* genes (Facklam et al., 2002). GAS is responsible for a wide variety of infections, including localized and systemic infections that can cause acute or chronic illness (Carapetis et al., 2005). In most cases, GAS causes pharyngitis (sore throat), tonsillitis, or skin infections such as impetigo. At times, GAS can cause severe and even life-threatening infections, such as necrotizing fasciitis and

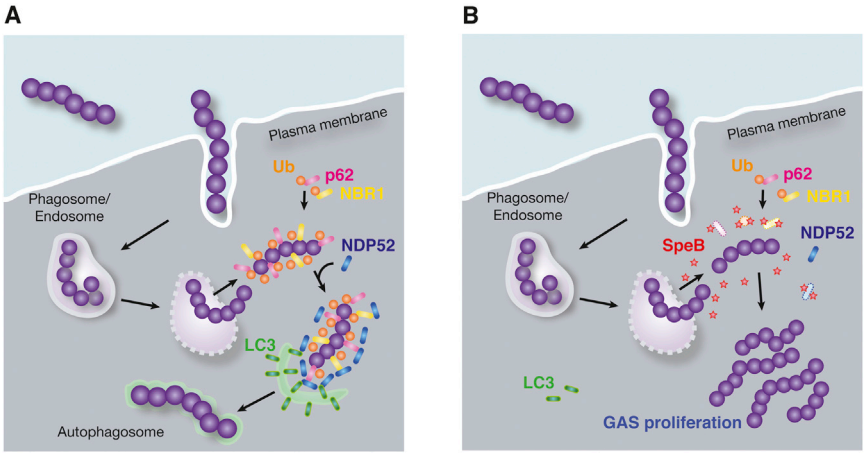


**Figure 1. Proposed Model for GAS-Induced Autophagy by M6 Strain and M1T1 Globally Disseminated Strain**

(A) Induction of autophagy by M6 GAS strain. GAS is engulfed by plasma membrane via the endocytic pathway, and GAS can escape from endosomes to the cytosol. However, cytosolic GAS is captured by autophagosomes through ubiquitylated adaptor recognition.

(B) Evading autophagy by globally disseminated M1T1 strain. M1T1 strain can produce SpeB within host cytosol, and SpeB degrades the ubiquitin-LC3 adaptor proteins, NDP52, p62, and NDR1.

bacteremia. Several streptococcal virulence factors, including pyrogenic exotoxins, streptokinase, and streptolysins, are thought to be involved in these diseases. Streptococcal toxic shock syndrome is a severe invasive infection that has recently been characterized by the sudden onset of shock and multiorgan failure; it has a high mortality rate, ranging from 30% to 70% (Bisno and Stevens, 1996). Whereas both host genetic susceptibility and variations in bacterial virulence factors play a key role in modulating disease manifestation, the responsible host factors and GAS genes have not been clarified. In addition, particular serotypes or *emm* types are reported to be more commonly associated with particular disease manifestations than others. However, serotypic designation does not always reflect the pathogenic potential of a given strain.

Whereas most GAS serotypes traditionally exhibit cyclic epidemiologic pattern, appearing and disappearing from the community at different times, the M1T1 subclones have persisted globally for more than 30 years as the most frequently isolated serotype from patients with invasive and noninvasive cases of GAS (Cole et al., 2006). The reemerged M1T1 clone differs from its ancestral M1 clone in several aspects. These global M1T1 clones have acquired new virulent genes via phage integration and show a high degree of variability in the expression of virulence genes. These complex factors may affect their unusual persistence, spread, and virulence.

Autophagy is a fundamental cellular homeostatic mechanism in which cytoplasmic constituents are engulfed by a characteristic double-membrane autophagosome, whose contents are eventually degraded in vacuoles or lysosomes. Autophagy was originally considered as a nonselective degradation system in response to starvation. However, it is now clear that autophagy can selectively degrade substrates, such as damaged organelles, excess peroxisomes, and aggregated proteins. In addition to cytoplasmic materials, invading pathogens such as *Salmonella enterica* serovar Typhimurium, *Listeria monocytogenes*, *Shigella flexneri*, and GAS can also be targeted for autophagy in a selective manner (Levine et al., 2011). In particular, serotype M6 GAS has been shown to be clearly captured and degraded by autophagy, so this infection model is widely used for selective autophagy (Nakagawa et al., 2004). Therefore, autophagy is now well recognized as a key immune mechanism against bacterial infections. While the exact mechanism by which bacteria are recognized and targeted for autophagy remains unclear, the best-characterized process involves ubiquitination (Shaid et al., 2013). Autophagy receptors, such as p62 (sequestosome 1 or SQSTM1), NBR1 (neighbor of BRCA1 gene 1), NDP52 (nuclear dot protein, 52 kDa), and OPTN (optineurin), are pattern recognition receptors, called sequestosome 1/p62-like receptors (SLRs), that recognize ubiquitylated substrates and recruit membranes for autophagosome formation through their interaction with ATG8 family proteins (Deretic, 2012). Some bacterial pathogens can evade autophagic degradation or otherwise subvert autophagy by various mechanisms. Newfound interactions of autophagy and pathogenic bacteria have revealed that autophagy may have different roles during distinct bacterial infections that, in addition to bacterial clearance, coordinate cell-autonomous signaling and in some cases even promote bacterial replication.

Investigating the globally disseminated M1T1 GAS clone, Barnett et al. (2013) now report that this GAS strain can not only survive, but can also replicate within epithelial cells, whereas the other serotype strains cannot (Figure 1). M1T1 GAS strains are the most frequently isolated serotype from patients with noninvasive and invasive infection. This observation indicates that the recent M1T1 GAS isolates may have acquired a unique survival ability compared with other serotypes. Consistent with this, Barnett et al. (2013) demonstrated that the replication of M1T1 GAS strain within epithelial cells correlated with the ability of the bacterium to avoid autophagy since the autophagosome marker LC3 (Atg8) did not colocalize with M1T1 GAS in infected cells. To check the localization of the autophagy-avoiding M1T1 GAS within infected cells, the authors used early and late endosomal marker staining and performed the transmitted electron microscopy (TEM). Strikingly, the authors found that the M1T1 GAS was able to replicate in the cytosol without being subject to autophagy, and the TEM observation indicated that M1T1 GAS is abundantly presented in the cytosol, whereas serotype M6 strain was contained within a membrane-bound autophagosome compartment. These observations are important because cytosolic GAS was believed to be targeted for selective autophagy (Nakagawa et al., 2004).

Next, the authors examined whether the M1T1 GAS could be recognized by

the ubiquitylation machinery or by the ubiquitin-LC3 adaptor proteins, NDP52, p62, or NBR1. Ubiquitylation is thought to be a critical step in selective autophagy against intracellular invading bacterial pathogens (Shaid et al., 2013). LC3 can directly target ubiquitylated bacteria via ubiquitin-LC3 adaptor proteins. Intracellular M6 GAS was found associated with NDP52, p62, and NBR1 in a time-dependent manner, and M6 GAS was efficiently targeted by the autophagy. In contrast, intracellular M1T1 GAS was not found in association with these adaptor proteins, indicating that the M1T1 GAS actively evades autophagic recognition and degradation.

To determine how the M1T1 GAS can evade the selective autophagy, Barnett et al. (2013) focused on a cysteine protease, SpeB, because the M6 strain (JRS4) was shown to be defective for SpeB expression. The streptococcal SpeB cysteine proteinase is one of the earliest identified secreted proteins from GAS. The speB gene is chromosomally encoded and is highly conserved in essentially all strains of GAS. The gene encodes the 40 kDa SpeBz protein that is autocatalytically processed into the 28 kDa SpeBm version (Nelson et al., 2011). Cysteine 192 in SpeBm needs to be reduced and forms a catalytic dyad with histidine 340 to be active. SpeB is a member of the C10 family of cysteine proteinases, which can be found in many pathogenic bacteria, including *Bacteroides*. Barnett et al. (2013) constructed a SpeB-defective mutant of M1T1 GAS and SpeB-expressing M6 GAS and compared their relative growth and resistance to autophagy. SpeB-defective mutant of M1T1 GAS was clearly targeted for autophagy, and SpeB-expressing M6 GAS could evade autophagy. These observations indicate that SpeB expression is crucial for GAS to avoid autophagy and thereby survive and replicate in the host cytosol.

Finally, Barnett et al. (2013) determined how SpeB confers resistance to autophagy. There are several possible mechanisms for this process. SpeB shows a broad-spectrum cysteine protease activity, and the specific substrates of SpeB within the cytosol are still unclear. Barnett et al. (2013) purified SpeB from M1T1 GAS and found that SpeB could degrade the host ubiquitin-LC3 adaptor proteins, NDP52, p62, and NBR1, as well as ubiquitylated proteins from epithelial cell extracts. Thus, GAS SpeB protease appears to be both necessary and sufficient to degrade ubiquitylation components within the host cytosol, resulting in evading autophagy.

The results described by Barnett et al. (2013) provide significant insight into the mechanism of autophagy escape by GAS. SpeB is thought to be an important virulence factor in GAS infection, and its activity has been adapted to widely affect host responses. To colonize and infect the human host, GAS needs to be able to counteract or avoid multiple aspects of human innate and adaptive immune responses. SpeB has diverse activities, including degradation of immunoglobulins, complement C3b, chemokines, and surface attachment molecules (Nelson et al., 2011). These proteolytic activities are important not only for the virulence of GAS, but also for bacterial survival strategies within the host. Recent comparative genomic studies indicate that GAS has lost several amino acid biosynthesis pathways during evolution, and therefore the activities of proteases are essential for the uptake of essential amino acids to support bacterial growth and proliferation. Thus, whereas the authors only showed the degradation of some ubiquitylated adaptor proteins within the host cell, SpeB has a very broad range of proteolytic substrates. Therefore, the degradation of other cellular components by SpeB may affect additional host cell functions, and these await discovery.

## REFERENCES

Barnett, T.C., Liebl, D., Seymour, L.M., Gillen, C.M., Lim, J.Y., LaRock, C.N., Davies, M.R., Schulz, B.L., Nizet, V., Teasdale, R.D., et al. (2013). Cell Host Microbe *14*, this issue, 675–682.

Bisno, A.L., and Stevens, D.L. (1996). N. Engl. J. Med. *334*, 240–245.

Carapetis, J.R., Steer, A.C., Mulholland, E.K., and Weber, M. (2005). Lancet Infect. Dis. *5*, 685–694.

Cole, J.N., McArthur, J.D., McKay, F.C., Sanderson-Smith, M.L., Cork, A.J., Ranson, M., Rohde, M., Itzek, A., Sun, H., Ginsburg, D., et al. (2006). FASEB J. *20*, 1745–1747.

Deretic, V. (2012). Curr. Opin. Immunol. *24*, 21–31.

Facklam, R.F., Martin, D.R., Lovgren, M., Johnson, D.R., Efstratiou, A., Thompson, T.A., Gowan, S., Kriz, P., Tyrrell, G.J., Kaplan, E., and Beall, B. (2002). Clin. Infect. Dis. *34*, 28–38.

Levine, B., Mizushima, N., and Virgin, H.W. (2011). Nature *469*, 323–335.

Nakagawa, I., Amano, A., Mizushima, N., Yamamoto, A., Yamaguchi, H., Kamimoto, T., Nara, A., Funao, J., Nakata, M., Tsuda, K., et al. (2004). Science *306*, 1037–1040.

Nelson, D.C., Garbe, J., and Collin, M. (2011). Biol. Chem. *392*, 1077–1088.

Shaid, S., Brandts, C.H., Serve, H., and Dikic, I. (2013). Cell Death Differ. *20*, 21–30.