Bacterial pathogens have evolved extraordinary mechanisms to efficiently infect host organisms. A majority of these pathogens do so by delivering virulence factors into host cells, which act to dampen host defenses or utilize the host as a niche for replication. Although regulation of virulence factor expression by bacterial pathogens is a well known pathogenic mechanism [1], the concept of host-protective virulence factors is emerging. Recently, several strategies by which pathogens appear to be attenuating their own lethality towards host cells have been documented, suggesting that increased hostility and damage of host cells is not necessarily beneficial to the pathogen. Virulence is often defined as the ability of a pathogen to inflict damage on host cells, and the following discussion addresses the concept that increased virulence is not always beneficial to the pathogen, and moderating it to preserve host cells is a mechanism several pathogens use as part of their overall pathogenic strategy. This strategy is well known for obligate intracellular pathogens, but has become an emerging theme in extracellular and facultative intracellular bacteria.

*Yersinia* spp., *Shigella flexneri*, *Helicobacter pylori*, and diarrheagentic *Escherichia coli* are well known for their ability to kill host cells. For *Yersinia*, death of infected macrophages dampens cytokine release and enables the pathogen to propagate with minimal challenges from the immune system [2]. Two recent studies suggest that cytotoxicity caused by *Yersinia* species is tightly regulated. *Yersinia pestis*, the etiologic agent of plague, and gastroenteritis-inducing *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* all encode a cytotoxic virulence factor called YopJ/P (YopJ in the two former species and YopP in the latter), which are translocated into infected cells via a type III secretion system (T3SS) [2,3]. Altering the cytotoxicity of *Y. pseudotuberculosis* affects its virulence. Decreased secretion of YopJ was shown to enhance *Y. pseudotuberculosis* pathogenesis in vivo [4]. Similarly for *Y. pestis*, enhanced cytotoxicity results in decreased incidence of plague in vivo [5]. Tight regulation of cytotoxicity by pathogenic *Yersinia* is an efficient virulence strategy. Increased apoptosis of infected immune cells decreases production of proinflammatory cytokines; however, some inflammation at the early stages of infection is thought to facilitate tissue damage necessary for movement of bacteria and infected cells to other sites of replication within the host [4].

Enteropathogenic *Escherichia coli*, enterohaemorrhagic *E. coli* (EPEC and EHEC, respectively), and *Citrobacter rodentium* are attaching and effacing (A/E) pathogens that cause severe diarrheagenic disease [6]. The ability of A/E pathogens to kill intestinal epithelial cells has been well documented [7–14]. The type III secreted (T3S) effector EspF has a role in host cell death by causing mitochondrial-dependent apoptosis [11,15]. We recently found that the T3S effector EspZ modulates cytotoxicity towards host cells. An EPEC *espZ* mutant (ΔEspZ) caused enhanced cytotoxicity in host cells when compared to the wild-type strain [16], which was surprising since the ΔEspZ strain is severely attenuated for virulence in vivo [17]. EspZ acts in part through the host transmembrane glycoprotein CD90 to activate focal adhesion kinase (FAK)-based survival pathways (Figure 1) [16]. Others found that the T3S effector NleH also dampens apoptosis of EPEC-infected cells, but via interaction with a Bcl-2-related protein involved in the mitochondrial death pathway (Figure 1) [18]. Unlike EspZ, NleH is not essential for EPEC colonization and only moderately impacts on A/E pathogen disease in vivo [19,20]; however, there are likely other host-protective virulence factors that act redundantly to NleH during EPEC infection.

*H. pylori* causes apoptosis of infected gastric epithelial cells [21]. Apoptosis induction by *H. pylori* has been linked to a secreted toxin called VacA, which induces cytochrome c release from mitochondria (Figure 1) [22]. Recently, it was determined that VacA-mediated apoptosis is counteracted by a type IV secreted (T4S) protein called CagA by both blocking pinocytosis of VacA and inhibiting VacA-mediated cytochrome c release from mitochondria [23] (Figure 1). Interestingly, loss of CagA in a VacA* H. pylori* strain decreases bacterial colonization and the incidence of gastric hyperplasia, adenocarcinoma, and inflammation [24]. Similar to the aforementioned pathogens, *H. pylori* has evolved a delicate interplay between host-protective and -detrimental virulence factors that are able to fine-tune virulence while promoting their propagation.

*S. flexneri*, the etiologic agent of bacillary dysentery, causes death of infected macrophages and epithelial cells [25]. Despite this, several host-protective strategies are employed by *S. flexneri*. The T3S effector OspE was recently found to enhance adhesion of infected host cells to the underlying extracellular matrix [26]. Whether OspE activates host cell survival pathways directly is unknown; however, its interaction with integrin-linked kinase

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Figure 1. Strategies evolved by bacterial pathogens to restrain virulence. (A) EPEC injects effector proteins into intestinal epithelial cells (IECs) via a T3SS. EspF localizes to mitochondria and causes release of cytochrome c into the host cell cytosol, which results in apoptotic death of the host cells. NleH interacts with Bax inhibitor-1 (BII-1), which inhibits release of cytochrome c from mitochondria. EspZ interacts with CD98, which then stimulates phosphorylation of focal adhesion kinase (FAK) to promote survival. Localization of NleH and EspZ in host cells during early stages of EPEC infection is unclear and has been portrayed as shown for simplicity. (B) *H. pylori* injects virulence factors into gastric epithelial cells via a type IV secretion system in addition to secreting soluble toxins. VacA is an *H. pylori*-secreted toxin that enters cells by pinocytosis and penetrates intracellular endosome trafficking pathways. VacA causes release of cytochrome c from mitochondria of infected cells, thus mediating host cell apoptosis. CagA is a T4S virulence factor, which prevents both pinocytosis/trafficking and cytochrome c release by VacA. Functions of CagA are dependent on its phosphorylation state, not depicted here. (C) *S. flexneri* enters IECs from their basolateral surface and then resides in the cell cytoplasm. Pro-survival signals are unknown. The observation that *S. flexneri* enters IECs from their basolateral surface and then resides in the cell cytoplasm suggests that protecting host cells may be a factor that is essential for its pathogenesis. The concept of host-protective virulence factors will become more apparent in other pathogenic strategies and may become interesting targets to combat bacterial disease. Importantly, virulence phenotypes that appear counterintuitive should not be ignored. Future studies into pathogenic mechanisms of virulent bacteria will likely reveal important roles for effectors or regulatory mechanisms that help the host cell and promote bacterial pathogenesis.

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References

1. Meikalanos JJ (1992) Environmental signals controlling expression of virulence determinants in bacteria. J Bacteriol 174: 1–7.
2. Aepfelbacher M, Trasak C, Rackesdchel K (2007) Effector functions of pathogenic *Yersinia* species. Thromb Haemost 98: 521–529.
3. Mills SD, Boland A, Sory MP, van der Smissen P, Kerkhofs C, et al. (1997) *Yersinia enterocolitica* induces apoptosis in macrophages by a process requiring functional type III secretion and translocation mechanisms and involving YopP, presumably acting as an effector protein. Proc Natl Acad Sci U S A 94: 12638–12643.
4. Brodsky IE, Medzhitov R (2008) Reduced secretion of YopJ by *Yersinia* limits in vivo cell death but enhances bacterial virulence. PLoS Pathog 4: e1000067. doi:10.1371/journal.ppat.1000067.
5. Zashburner A, Telihar A, Levy Y, Bar-Haim E, Halperin G, et al. (2009) *Yersinia pestis* endowed with increased cytotoxicity is avirulent in a bubonic plague model and induces rapid protection against pneumonic plague. PLoS ONE 4: e5930. doi:10.1371/journal.pone.0005938.
6. Cronen MA, Finlay BB (2010) Molecular mechanisms of Escherichia coli pathogenicity. Nat Rev Microbiol 8: 26–38.
7. Vallance BA, Deng W, Jacobson K, Finlay BB (2003) Host susceptibility to the attaching and effacing bacterial pathogen *Citrobacter rodentium*. Infect Immun 71: 3443–3453.
8. Vallance BA, Deng W, Knodler LA, Finlay BB (2002) Mice lacking T and B lymphocytes develop...
transient colitis and crypt hyperplasia yet suffer impaired bacterial clearance during Citrobacter rodentium infection. Infect Immun 70: 2070–2081.

9. Abul-Milh M, Wu Y, Lau B, Lingwood CA, Barnett Foster D (2001) Induction of epithelial cell death including apoptosis by enteropathogenic Escherichia coli expressing bundle-forming pili. Infect Immun 69: 7356–7364.

10. Crane JK, Veizina CM (2005) Externalization of host cell protein kinase C during enteropathogenic Escherichia coli infection. Cell Death Differ 12: 113–127.

11. Crane JK, McNamara BP, Donnenberg MS (2001) Role of EspF in host cell death induced by enteropathogenic Escherichia coli. Cell Microbiol 3: 197–211.

12. Flynn AN, Buret AG (2008) Caspases-3, -8, and -9 are required for induction of epithelial cell apoptosis by enteropathogenic E. coli but are dispensable for increased paracellular permeability. Microb Pathog 44: 311–319.

13. Barnett Foster D, Abul-Milh M, Huesca M, Lingwood CA (2006) Enterohemorrhagic Escherichia coli induces apoptosis which augments bacterial binding and phosphatidylethanolamine exposure on the plasma membrane outer leaflet. Infect Immun 74: 3109–3115.

14. Crane JK, Majumdar S, Pickhardt DF, 3rd (1999) Host cell death due to enteropathogenic Escherichia coli has features of apoptosis. Infect Immun 67: 2575–2584.

15. Nougayrede JP, Donnenberg MS (2004) Enteropathogenic Escherichia coli EspF is targeted to mitochondria and is required to initiate the mitochondrial death pathway. Cell Microbiol 6: 1097–1111.

16. Shames SR, Deng W, Gutman JA, de Hoog CL, Li Y, et al. (2010) The pathogenic E. coli type III effector EspZ interacts with host CD90 and facilitates host cell prosurvival signaling. Cell Microbiol 12: 1322–1339.

17. Deng W, Puente JL, Gruenheid S, Li Y, Vallance BA, et al. (2004) Dissecting virulence: systematic and functional analyses of a pathogenicity island. Proc Natl Acad Sci U S A 101: 3597–3602.

18. Hemrajani C, Berger CN, Robinson KS, Marches O, Mousnier A, et al. (2010) NleH effectors interact with Bax inhibitor-1 to block apoptosis during enteropathogenic Escherichia coli infection. Proc Natl Acad Sci U S A 107: 3129–3134.

19. Garcia-Angulo VA, Deng W, Thomas NA, Finlay BB, Puente JL, et al. (2008) Regulation of expression and secretion of NleH, a novel locus of enterocyte effacement-encoded effector in Citrobacter rodentium. J Bacteriol 190: 2388–2399.

20. Hemrajani C, Marches O, Wiles S, Girard F, Dennis A, et al. (2008) Role of NleH, a type III secreted effector from attaching and effacing pathogens, in colonization of the bovine, ovine, and murine gut. Infect Immun 76: 4804–4813.

21. Moss SF, Calam J, Agarwal B, Wang S, Holt PR (1996) Induction of gastric epithelial apoptosis by Helicobacter pylori. Gut 38: 498–501.

22. Galimiche A, Rassow J, Doye A, Cagnol S, Chambard JC, et al. (2000) The N-terminal 34 kDa fragment of Helicobacter pylori vacuolating cytotoxin targets mitochondria and induces cytochrome c release. EMBO J 19: 6361–6370.

23. Oldani A, Cormont M, Hofman V, Chiozzi V, Oregioni O, et al. (2009) Helicobacter pylori counteracts the apoptotic action of its VacA toxin by injecting the CagA protein into gastric epithelial cells. PLoS Pathog 5: e1000603. doi:10.1371/journal.ppat.1000603.

24. Franco AT, Johnston E, Krishna U, Yamaoka Y, Israel DA, et al. (2008) Regulation of gastric carcinogenesis by Helicobacter pylori virulence factors. Cancer Res 68: 379–387.

25. Carneiro LA, Travassos LH, Sours F, Taitoli I, Magalhaes JG, et al. (2009) Shigella induces mitochondrial dysfunction and cell death in nonmyleoid cells. Cell Host Microbe 5: 123–136.

26. Kim M, Ogoawa M, Fujita Y, Yoshikawa Y, Nagai T, et al. (2009) Bacteria hijack integrin-linked kinase to stabilize focal adhesions and block cell detachment. Nature 459: 578–582.