Bacterial toxins and the immune system: show me the in vivo targets

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Microorganisms that cause persistent infection often exhibit specific adaptations that allow them to avoid the adaptive immune response. Recently, several bacterial toxins have been shown in vitro to disrupt immune cell functions. However, it remains to be established whether these activities are relevant during infection and whether these toxins have specifically evolved to disrupt the adaptive immune system.

Bacterial pathogens, particularly those that have coevolved extensively with their hosts, possess a vast array of fine-tuned determinants that are capable of modulating a variety of cellular functions. Often described as "toxins," "modulins," or "effectors," these bacterial products posses precise biochemical activities, which allow them to stimulate or interfere with a variety of cellular processes (1). Delivery of these bacterial effectors of virulence is mediated by an equally diverse set of specific adaptations. These adaptations include accessory proteins (referred to as toxin "B" subunits) that target specific receptors and vesicular trafficking pathways to deliver the enzymatically active components (known as toxin "A" subunits) to the appropriate cellular location (2), as well as more complex organelles that act as injection devices for the direct delivery of the toxins (such as the type III and type IV secretion systems; reference 3). Bacterial toxins have attracted a lot of attention from scientists, particularly those toxins whose powerful effects have conferred for them a special place in the annals of microbiology, such as the botulinum, diphteria, and anthrax toxins. Consequently, the understanding of the biochemical activities of bacterial toxins has traditionally been far ahead of the understanding of the pathogenesis of the microorganisms that produce them.

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When investigating a bacterial toxin, it is important to consider its biochemical activity as well as its potential target(s) within the host cell and potential target cell(s) within the host. It is also important to consider its function in the context of the toxin's role during the pathogenesis of the bacterium that produces it. The field of toxinology has been remarkably successful in identifying the biochemical activities of many bacterial toxins and reasonably successful at defining some of their potential targets within the host cell. Protein toxins that exert their activity as proteases, phosphatases, ADP ribosylases, adenylate cyclases, guanine nucleotide exchange factors, GTPase–activating proteins, and nucleases have been described (2, 4–6). In addition, in many instances the intracellular targets of these biochemical activities have been identified. However, identifying the cell type(s) targeted during infection and establishing the actual role of these toxins in pathogenesis is difficult. Consequently, little is known about these important aspects of toxin biology. This is even the case for toxins whose biochemical mechanism has been known for many years. Notable exceptions are toxins such as tetanus and botulinum toxin, which cause such a distinct neurological pathology in vivo that the identification of the relevant cellular targets was greatly facilitated (7).

Targeting T cells

In this issue of the *JEM*, two articles describe a potential role for two bacterial toxins in the modulation of the immune system by interfering with T cell signaling. Using an in vitro Jurkat T cell system, Gerke et al. (8) report that *Yersinia pseudotuberculosis* inhibits T cell activation and that the inhibitory activity was strictly dependent on the *Yersinia* outer protein H (YopH), a potent tyrosine phosphatase that is delivered to host cells by a bacterially encoded type III protein secretion system. Previous studies by a number of laboratories have identified several tyrosine phosphorylated proteins as apparent targets for the tyrosine phosphatase activity of YopH in cell culture systems. These include p130Cas, focal adhesion kinase, and paxillin in HeLa cells, and Fyb/SLAP130, p130Cas, and SKAP55 in macrophages (9–12). In general, these are proteins involved in integrin signaling, which is consistent with the proposed role of YopH as an antiphagocytic molecule. More recent studies have shown that YopH can also inhibit T cell signaling in-vitro and that this activity could be correlated with the presence of YopH and the dephosphorylation of the tyrosine kinase Lck (13). Gerke et al. (8) have now extended those studies and added the T cell–signaling adaptor proteins LAT and SLP-76 to the list of potential YopH substrates. These adaptor proteins are essential for the transduction of signals from the T cell receptor and its associated tyrosine kinases, and their tyrosine phosphorylation is required for their activities. Consequently, Gerke et al. (8) argue that tyrosine dephosphorylation of these adaptor proteins by YopH is responsible for the observed inhibition of T cell receptor signaling after *Yersinia* infection of cultured Jurkat T cells. They also present data that argue that both of these proteins are high affinity substrates of YopH, presumably requiring small amounts of translocated toxin to be targeted for dephosphorylation.

In the second article, Rossi Paccani et al. (14) report that anthrax toxin also disrupts T cell signaling but by a completely different mechanism. Anthrax toxin, which is produced by *Bacillus anthracis*, is composed of two enzymatic or A subunits known as edema factor (EF) and lethal factor (LF) that alternatively associate with a B subunit, known as protective antigen, which mediates their delivery into target cells (15). EF is a potent calcium/calmodulin–dependent adenylate cyclase, which causes a massive increase of cyclic AMP in intoxicated cells and global disruption of cell signaling. LF is a zinc–dependent protease that specifically disrupts the mitogen–activated protein (MAP) kinase signaling pathways by cleaving the activating MAP kinase kinases MEK1/2 and MKK3, MKK4, MKK6, and MKK7 (16–19). Rossi Paccani et al. (14) showed that treatment of peripheral blood lymphocytes with protective antigen in combination with either LF or EF effectively blocked T cell signaling as measured by the expression levels of the surface activation markers CD69 and CD25, the production of cytokines, and cell proliferation. Given the ubiquitous presence of the anthrax toxin receptors and the essential role for MAP kinases and cAMP in T cell signaling, the results presented by Rossi Paccani et al. (14) are expected.

In vivo targets

The broader issue raised by these two and other studies with other bacterial toxins that disrupt immune cell function in vitro, however, is the in vivo relevance of the findings. Have these toxins evolved to specifically target T cell signaling? Do these pathogens specifically interfere with T cell signaling during the course of an infection? Unfortunately, in vitro studies, although useful to provide testable hypotheses, cannot provide answers to these important questions.

In the case of YopH, Gerker et al. (8) make a strong case for SLP-76 and LAT as high affinity substrates for this tyrosine phosphatase and hence of potential relevance during infection when pre-sumably the pathogen would deliver small quantities of this toxin. Indeed, it is often the case that under vitro experimental conditions, cells are exposed to toxin quantities not usually delivered during actual infections. Nevertheless, it is not known how much YopH is delivered by *Yersinia* during infection, and there is no evidence demonstrating that *Yersinia* hampers T cell function during infection. *Yersinia* infections are usually acute (20), arguing against the need for these pathogens to counteract the host-specific adaptive immune responses during infection. However, although rare, chronic infections are sometimes observed (21, 22), and in those cases, the ability to interfere with T cell function might be useful to the pathogen. Whether this function of YopH has been specifically selected by evolution or is simply a "by-product" of another of its demonstrated functions, such as disruption of macrophages during acute infection, remains to be established.

Anthrax toxin possesses even a more difficult challenge because its biochemical activity (such as inhibition of MAP kinase signaling) can potentially affect a large number of cellular processes. Therefore, the ability of anthrax toxin to inhibit MAP kinase signaling can result in the in vitro inhibition of any of the rather large number of intracellular signaling pathways that involve these kinases, regardless of their relevance during infection. For example, if added to cultured neurons, anthrax toxin would certainly prevent neurite outgrowth, a process strictly dependent on MAP kinase signaling (23). However, it would be hard to argue that this is a relevant function for the toxin during infection. By the same token, anthrax toxin predictably inhibited in vitro T cell signaling, a process strictly dependent on MAP kinase signaling. However, more experiments will be required to establish whether this activity of the toxin is important during infection. If untreated, *B. anthracis* infections are usually hyper acute (24); therefore, inhibiting T cell signaling may not provide a significant advantage to the pathogen. On occasions, *B. anthracis* can be associated with sub–acute infections (such as cutaneous anthrax; reference 24), in which case inhibition of T cell function may provide some advantages to the pathogen. In any case, because *B. anthracis* is most likely an "accidental" pathogen of mammals, it is unlikely that evolution may have played any role in directly shaping the effects of anthrax toxin on T cells. Therefore, this activity might be more a byproduct, relevant or not, of other activities of this toxin in the normal ecology of *B. anthracis*.

Although every pathogen must contend with the onslaught of the innate immune responses, it is not necessarily the case that every pathogen must counteract the acquired immune response to fulfill its replication program. For most pathogens that cause acute infections, their life cycle within the host is most often over by the time the naive host mounts a meaningful acquired immune response capable of controlling the infection. In fact, most infections with pathogens that have co-evolved with their hosts are indeed asymptomatic, do not lead to overt harm, and most often result in protective convalescent immunity. On the other hand, pathogens that cause persistent infections might be under strong evolutionary pressure to evolve specific mechanisms to avoid acquired immune responses. Indeed, mechanisms of antigenic variation or specific inhibition of antigen presentation evolved by microbial pathogens are well documented (25). For example, many viral pathogens specifically interfere, by a variety of mechanisms, with both major histocompatibility class I and II antigen–presenting pathways (26, 27). In addition, many viral, bacterial, and protozoan pathogens undergo rapid antigenic variation to evade the onslaught of the acquired immune response (28–30). Whether inhibition of T cell signaling by anthrax or YopH toxins should be added to the list of pathogenic mechanisms specifically evolved to counter the acquired immune response awaits further in vivo experimentation.

REFERENCES

1. Galán, J.E. 2002. The cell biology of microbial infections: coming of age. *J. Cell Biol.* 158:387–388.

2. Alouf, J. 2000. Bacterial protein toxins: an overview. *Methods Mol. Biol.* 145:1–26.

3. Galán, J.E., and A. Collmer. 1999. Type III secretion machines: bacterial devices for protein delivery into host cells. *Science.* 284:1322–1328.

4. Aktories, K. 1997. Bacterial toxins that target Rho proteins. *J. Clin. Invest.* 99:827–829.

5. Barbieri, J., M. Riese, and K. Aktories. 2002. Bacterial toxins that modify the actin cytoskeleton. *Annu. Rev. Cell Dev. Biol.* 18:315–344.

6. Lara-Tejero, M., and J.E. Galán. 2000. A bacterial toxin that controls cell cycle progression as a deoxyribonuclease I-like protein. *Science.* 290:354–357.

7. Schiavo, G., M. Matteoli, and C. Montecucco. 2000. Neurotoxins affecting neuroexocytosis. *Physiol. Rev.* 80:717–766.

8. Gerke, C., S. Falkow, and Y.-h. Chien. 2005. The adaptor molecule LAT and SLP-76 are specifically targeted by *Yersinia* to inhibit T cell activation. *J. Exp. Med.* 201:361–371.

9. Black, D.S., A. Marie-Cardine, B. Schraven, and J.B. Bliska. 2000. The *Yersinia* tyrosine phosphatase YopH targets a novel adhesion-regulated signalling complex in macrophages. *Cell Microbiol.* 2:401–414.

10. Black, D.S., and J.B. Bliska. 1997. Identification of p130Cas as a substrate of *Yersinia* YopH (Yop51), a bacterial protein tyrosine phosphatase that translocates into mammalian cells and targets focal adhesions. *EMBO J.* 16:2730–2744.

11. Hamid, N., A. Gustavsson, K. Andersson, K. McGee, C. Persson, C.E. Rudd, and M. Fallman. 1999. YopH dephosphorylates Cas and Fyn-binding protein in macrophages. *Microb. Pathog.* 27:231–242.

12. Persson, C., N. Carballeira, H. Wolf-Watz, and M. Fallman. 1997. The PTPase YopH inhibits uptake of *Yersinia*, tyrosine phosphorylation of p130Cas and FAK, and the associated accumulation of these proteins in peripheral focal adhesions. *EMBO J.* 16:2307–2318.

13. Alonso, A., N. Bottini, S. Bruckner, S. Rahmouni, S. Williams, S. Schoenberger, and T. Mustelin. 2004. Lck dephosphorylation at Tyr-394 and inhibition of T cell antigen receptor signaling by *Yersinia* phosphatase YopH. *J. Biol. Chem.* 279:4922–4928.

14. Rossi Paccani, S., F. Tonello, R. Ghittoni, M. Natale, L. Muraro, M.M. D'Elios, W.-J. Tang, C. Montecucco, and C.T. Baldari. 2005. Anthrax toxins suppress T lymphocyte activation by disrupting antigen receptor signaling. *J. Exp. Med.* 201:325–331.

15. Collier, R., and J. Young. 2003. Anthrax toxin. *Annu. Rev. Cell Dev. Biol.* 19:45–70.

16. Duesbery, N.S., C.P. Webb, S.H. Leppla, V.M. Gordon, K.R. Klimpel, T.D. Copeland, N.G. Ahn, M.K. Oskarsson, K. Fukasawa, K.D. Paull, and G.F. Vande Woude. 1998. Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor. *Science.* 280:734–737.

17. Chopra, A.P., S.A. Boone, X. Liang, and N.S. Duesbery. 2003. Anthrax lethal factor proteolysis and inactivation of MAPK kinase. *J. Biol. Chem.* 278:9402–9406.

18. Park, J.M., F.R. Greten, Z.W. Li, and M. Karin. 2002. Macrophage apoptosis by anthrax lethal factor through p38 MAP kinase inhibition. *Science.* 297:2048–2051.

19. Vitale, G., L. Bernardi, G. Napolitani, M. Mock, and C. Montecucco. 2000. Susceptibility of mitogen-activated protein kinase kinase family members to proteolysis by anthrax lethal factor. *Biochem. J.* 352:739–745.

20. Bottone, E.J. 1999. *Yersinia enterocolitica*: overview and epidemiologic correlates. *Microbes Infect.* 1:323–333.

21. Hoogkamp-Korstanje, J.A., J. de Koning, and J. Heesemann. 1988. Persistence of *Yersinia enterocolitica* in man. *Infection.* 16:81–85.

22. Segal, R. 2003. Selectivity in neurotrophin signaling: theme and variations. *Annu. Rev. Neurosci.* 26:299–330.

23. Mock, M., and A. Fouet. 2001. Anthrax. *Annu. Rev. Microbiol.* 55:647–671.

24. Hilleman, M. 2004. Strategies and mechanisms for host and pathogen survival in acute and persistent viral infections. *Proc. Natl. Acad. Sci. USA.* 101:14560–14566.

25. Früh, K., K. Ahn, and P.A. Peterson. 1997. Inhibiton of MHC class I antigen presentation by viral proteins. *J. Mol. Med.* 75:18–27.

26. Hedge, N.R., M.S. Chevalier, and D.C. Johnson. 2003. Viral inhibition of MHC class II antigen presentation. *Trends Immunol.* 24:278–285.

27. Hahn, Y.S. 2003. Subversion of immune responses by hepatitis C virus: inunomodulatory strategies beyond evasion? *Curr. Opin. Immunol.* 15:443–449.

28. Palmer, G., W. Brown, and F. Rurangirwa. 2000. Antigenic variation in the persistence and transmission of the ehrlichia *Anaplasma marginale. Microbes Infect.* 2:167–176.

29. Brunham, R., F. Plummer, and R. Stephens. 1993. Bacterial antigenic variation, host immune response, and pathogen–host coevolution. *Infect. Immun.* 61:2273–2276.

30. Zambrano-Villa, S., D. Rosales-Borjas, J.C. Carrero, and L. Ortiz-Ortiz. 2002. How protozoan parasites evade the immune response. *Trends Parasitol.* 18:272–278.