Escherichia coli K1 meningitis: Analysis of the effects of CNF1 toxin in newborn mice questions its virulence function

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Bacterial virulence factors and toxins are usually considered to be precision-guided weaponry used by bacteria to hijack host cellular processes for the profit of the pathogen. In this issue of Virulence, Chang and colleagues question this view by exploring the role of the CNF1 toxin of Escherichia coli K1 strains that are associated with neonatal meningitis. CNF1 is found in 10% of the E. coli K1 strains that can cause lethal meningitis or neurological sequelae in newborns and infants. The CNF1 toxin is a bacterial enzyme that targets the host cell RhoGTPases that are essential elements of host cell homeostasis and host defense against pathogens. The activity of RhoGTPases is critical for cell migration and phagocytosis, as well as for reactive oxygen species production and the innate immune response. It is probably for these reasons that more than 30 virulence factors expressed by various pathogens target RhoGTPases. Recently, RhoGTPases have emerged as evolutionarily conserved host sensors that are able to gauge the pathogenic potential of microbes, enabling the host to detect and react to the CNF1-expressing E. coli. In this context, the role of CNF1 during infection requires a careful analysis that accounts for both the bacteria and the host innate immune response. In this issue, Chang and colleagues analyzed the effects of CNF1 expression in the uptake of E. coli K1 by macrophages and the development of meningitis in newborn mice. Interestingly, their results provide a new example of the complexity of the effects of CNF1 during infection, likely reflecting both its virulence potential and its detection by the innate immune system.

To determine the effects of CNF1 on the uptake of E. coli K1 by macrophages, Chang and colleagues took advantage of a gentamicin protection assay using macrophages. They show that the E. coli K1 strain E44 expressing CNF1 had a reduced capacity to enter into macrophages compared with that of an isogenic CNF1-impaired strain. They also observed that CNF1 triggered massive actin remodeling of macrophages. The authors linked both observations and hypothesized that CNF1 hijacks cellular actin cytoskeleton to limit the availability of free actin for bacterial invasion. Because the adhesin OmpA has been shown to be important for bacterial entry into brain microvascular endothelial cells and in macrophages, the authors suggest that CNF1 counteracts OmpA-mediated bacterial invasion. This hypothesis is interesting because it highlights the underappreciated interplay between OmpA and CNF1, although this interplay remains to be proven. The interplay between the virulence factors of pathogenic bacteria is fascinating because such interplay potentially increases the virulence repertoire of a strain by combining the effects of virulence factors. Indeed, an exciting hypothesis is that pathogenic strains adapt the combination of virulence factors to the required effect and to the cell type being targeted. Regarding CNF1 and OmpA, it would be interesting to analyze the expression and activity of both factors during the contact of E. coli with epithelial cells compared with macrophages. Evidence from an in vivo analysis of uropathogenic E. coli (UPEC) strains expressing CNF1 have demonstrated the interplay of CNF1 with α-hemolysin toxin when E. coli is in contact with macrophages. Taken together, these discoveries highlight the importance of analyzing virulence factors in their natural context, using the whole bacterium and taking into account the complex interplay of virulence factors that occurs during infection.

Interestingly, the authors’ observation of the reduced capacity of E. coli K1 expressing CNF1 to enter into macrophages compared with the isogenic CNF1-impaired strain is the opposite result to what has been observed in human brain microvascular endothelium. Furthermore, the invasive role of CNF1 has also been established using UPEC in the context of uroepithelial cells.
of these results would favor a cell-type specific effect of the CNF1 toxin that would trigger bacterial entry into epithelial cells and block phagocytosis by specialized immune cells. However, in vivo measurements of the bacterial load in the brain revealed a lower level of *E. coli* expressing CNF1 in the brain of infected mice, and mice infected with *E. coli* expressing CNF1 had an increased rate of survival (92% survival with the E44 strain, 73% survival with the isogenic ΔCNF1 strain, and 100% survival with the ΔCNF1 strain complemented with a CNF1-expressing plasmid). These data favor the hypothesis of a host protective role of CNF1 during infection. Surprisingly, despite a lower capacity of CNF1-expressing bacteria to invade the brain of newborn mice and an increased survival rate of the host, the authors show that the strains expressing CNF1 induced more pathological lesions in the brain. These data appear to be counter-intuitive unless the lesions in the brain are a detrimental side effect of a host protective inflammatory reaction. Supporting the hypothesis of CNF1 modulation of the inflammatory reaction in this model, IL-1β and TNFα cytokine levels were significantly different in the blood of mice depending on the presence or absence of CNF1 in the infecting strain. Further experiments will be needed to address the molecular and cellular questions of the global protective role of CNF1 during meningitis, but that is also detrimental to the brain integrity. In the context of CNF1-expressing strains specifically and in general for *E. coli* K1 meningitis, major progress would likely come from the identification of molecules that specifically block the detrimental inflammation in the brain but still allow for the beneficial effects of the immune response.

In conclusion, the work of Chang and colleagues on the role of CNF1 in *E. coli* K1 meningitis forces us to be humble with regards to the remaining knowledge we still need to acquire to fully understand the roles of virulence factors, their interplay, and their impact on disease processes.

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