Rethinking cholera pathogenesis- No longer all in the same “camp”

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The model whereby cholera toxin evokes chloride, and thus fluid, secretion by the intestinal epithelium is one of the most enduring paradigms in gastrointestinal physiology. Following internalization, the active subunit of cholera toxin, CtxA, is trafficked to the basolateral membrane and evokes ADP-ribosylation of the \( \alpha \)-subunits of stimulatory G proteins. In turn, this persistently activates adenyl cyclase, massively elevates cAMP, and triggers chloride secretion via the activation of CFTR chloride channels. Water follows paracellularly, leading to profuse watery diarrhea. The model has been expanded over the years to incorporate an additional effect of cholera toxin on the enteric nervous system, which amplifies the diarrheal response. Nevertheless, the prevailing wisdom has been that \( V. \) cholerae, unlike invasive diarrheal pathogens, evokes diarrhea by non-inflammatory means that leave the epithelium largely intact.

In their recent study published in \textit{Virulence}, Satitsri \textit{et al.} challenge this prevailing wisdom, at least for one cholera variant. \textit{V. cholerae} O1 El Tor variant (EL) is a major epidemic strain to which several recent large outbreaks of severe diarrheal illness have been attributed. Compared to classical (CL) and El Tor (ET) biotypes of \textit{V. cholerae} serotype O1, infection with the mixed biotype EL results in more severe diarrhea and dehydration, an effect that had previously been attributed to its much higher production of cholera toxin. Using a mouse model, Satitsri and colleagues set out to examine whether the increased severity of EL-associated disease additionally reflected an inflammatory component that might damage epithelial integrity.

Initial studies showed that inoculation of closed ileal loops in mice with EL bacteria resulted in a fluid accumulation response that was fundamentally distinct from that caused by CL bacteria. Whereas the response to CL was fully abrogated by a CFTR inhibitor, that evoked by EL involved not only CFTR, but also calcium-activated chloride channels (CaCC) as demonstrated with a CaCC inhibitor. Furthermore, EL, but not CL, infection compromised epithelial barrier function in a manner that was independent of the effect of infection on either CFTR or CaCC. EL, but not CL, infection also caused activation of NF-\( \kappa \)B, the production of proinflammatory cytokines, and increased expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). The ability of EL to activate NF-\( \kappa \)B, iNOS and COX-2 was linked to the effect of infection on epithelial barrier function, whereas COX-2 activation contributed to fluid secretion via the production of E-series prostaglandins that can elevate both cAMP and calcium in the epithelium. The authors also showed that both fluid accumulation and barrier dysfunction induced by EL infection could be reversed, in part, by an antibody to the LPS receptor, TLR-4. Finally, the authors studied whether the ability of EL to evoke inflammatory changes and barrier dysfunction is simply attributable to an increased capacity for Ctx production. While purified Ctx caused both fluid accumulation and barrier dysfunction in murine ileal loops, the former effect occurred at much lower toxin concentrations, and the ability of purified Ctx to cause barrier dysfunction was less than that induced by \textit{bona fide} EL infection, even when infection resulted in similar toxin concentrations. Further, Ctx alone had no effect on iNOS or COX-2 expression, whereas both proteins were potently stimulated by LPS. Thus, EL bacteria likely also stimulate Ctx-independent pathways to amplify mucosal inflammation and resulting barrier dysfunction, although it cannot be excluded that the kinetics and magnitude of toxin production in the setting of infection may not be modeled well by a one-time inoculation with purified toxin, and that these factors might influence the pathophysiological consequences that ensue with live infection.

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In summary, Satitsri and co-workers propose a model whereby EL infection causes an initial increase in epithelial permeability in the gut, which permits access of endogenous LPS to basolateral TLR4 and stimulation of NF-κB in intestinal epithelial cells, as well as the activation of subepithelial immunocytes. The resulting upregulation of iNOS and COX-2 further exacerbates barrier dysfunction, while COX-2-derived PGE₂ can trigger chloride secretion via both cAMP- and calcium-dependent pathways, and activation of CFTR and CaCC, respectively. These latter effects synergistically augment the cAMP-dependent prosecretory effects of Ctx itself, resulting in more severe diarrhea and dehydration that is further exacerbated by “leak-flux” diarrhea across the compromised epithelium.

This new contribution to the literature, therefore, sheds light on the complex mechanisms of diarrheal pathogenesis caused by emerging biotypes of an ancient pathogen. In fact, it is increasingly being recognized that Ctx can cause epithelial barrier dysfunction in vivo and in vitro, and that human cholera patients do in fact present with evidence of epithelial damage and activation of an innate immune response during the acute phase of the disease. Thus, particularly in the setting of infection with El Tor variants of V. cholerae, which in fact have become predominant causes of modern epidemics, we must entertain a more nuanced understanding of the mechanisms that result in life-threatening disease. These more complex mechanisms, moreover, may be overlaid on host-specific factors, such as variations in innate immune reactivity and perhaps also the influence of the highly variable resident microbiota, which is known to modulate epithelial function. This could account for heterogeneity in the clinical presentation of cholera, even in populations similarly exposed to pathogenic strains. Further, in the face of emerging antibiotic resistance, limiting therapeutic options for severe disease, new appro-a-ches to the treatment of cholera beyond supportive rehydration will likely be needed. In this regard, the work presented highlights possible targets, such as EP and TLR4 receptors, as well as NF-κB activation, that might profitably be explored in clinical trials if the current results can be extrapolated to human patients.

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