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We are excited to present our latest results on pathogen communication in the context of the human intestinal microbiome. To unravel these complex interactions and their effects on human health, we investigated an intriguing three-way interaction between pathogens, the microbiome, and metabolites ([URL or DOI here]).

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Enteropathogenic *Escherichia coli (EPEC)* virulence depends on the type III secretion system (T3SS), which transports effectors into host cells.

[Insert - I suggest a relatively simple model of the T3SS.]



<https://www.people.ku.edu/~rdguzman/Images/typeIIIcartoonBsaL5a.gif>

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In our paper we discovered that concentrations of >300 micromolar indole, a metabolite produced by species of the microbiome, strongly reduce the T3SS activity of EPEC and hence its virulence.

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To examine the effect of indole under hyper-virulence conditions, we utilized a co-culture model of EPEC and *Vibrio cholerae*. We previously showed that such co-culture enhances EPEC virulence by sensing the *V. cholerae* effector CAI-1. So, when we co-cultivated the two species, we found that increased T3SS activity in EPEC is CAI-1 dependent.

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We examined the effect of indole (500 uM) in the co-culture model and found complete inhibition T3SS activity. Combined with careful control experiments, we concluded that indole not only acts as a strong inhibitor but also impairs the upregulation of EPEC T3SS activity in response to *V. cholerae*.

[Insert – suggest an image from Fig. 3]

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While CAI-1 upregulates the transcription of T3SS-related genes, in the presence of indole, T3SS-related gene transcription was reduced. At 1:10 (indole:CAI-1), transcription was almost completely inhibited, indicating that indole inhibits type III secretion transcriptionally.

[Insert – image from Fig. 4]

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To determine if indole, produced by bacterial species have similar effect we added supernatant from the commensal bacterium, *B. thetaiotaomicron,* which converts tryptophan to indole. When added to co-cultures of EPEC and *V. cholerae*, the supernatant resulted in the inhibition of T3SS.

[Insert – suggest an image from Fig. 5]

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To understand the potentially complex interactions in the gut, we multi-cultured *B.* *thetaiotaomicron, V. cholerae*, and EPEC in various combinations. As expected, the co-culture of EPEC and *V. cholerae* showed elevated T3SS activity compared to EPEC alone. A triculture showed inhibition of T3SS activity similar to 500uM indole addition.

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Importantly, a tri-culture with a *B. thetaiotaomicron* mutant strain unable to produce indole, had strong T3SS activity as the co-culture. Based on these results we concluded that microbiome-derived indole interferes with EPEC T3SS activity and pathogen communication*.*

[Insert – suggest an image from Fig. 5. Or maybe a cartoon to describe the interactions.]

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So overall, we found that indole, exogenous or from *B. thetaiotaomicron,* interferes with communication between EPEC and *V. cholerae,* resulting in altered virulence and reduced pathogenicity.

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Our results may explain individual variability toward bacterial infections, which may be due to variations in intestinal indole. The indole concentration depends upon the microbiome composition and interbacterial communication. Indole then modulates type III secretion, which impacts the infection of human cells.

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Lots of implications! Altering microbiome composition could reduce infection, highlighting the need to understand better the complex network of inter-microbiome interactions and human-microbiome interactions. Also, eating protein-rich food may increase intestinal indole, reducing the incidence of infection.

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We appreciate the effort of our team and others who contributed!