



# mSphere of Influence: Combining Host and Pathogen Genetics To Disrupt Chronic Infections

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**ABSTRACT** Andrew J. Olive works in the field of host responses to chronic infections. In this mSphere of Influence article, he reflects on how “Tryptophan biosynthesis protects mycobacteria from CD4 T-cell-mediated killing” (Y. J. Zhang, M. C. Reddy, T. R. Ioerger, A. C. Rothchild, et al., *Cell* 155:1296–1308, 2013, <https://doi.org/10.1016/j.cell.2013.10.045>) impacted his own work using genetic approaches to dissect the interface between host and pathogen.

**KEYWORDS** *Mycobacterium*, *Mycobacterium tuberculosis*, genetics, immune evasion, persistent infections, transposon sequencing

During infection, *Mycobacterium tuberculosis* can evade and withstand the stresses imposed by the host immune response (1, 2). Understanding the precise mechanisms used by *M. tuberculosis* to survive an ongoing immune response in the host remains key to effectively developing new treatment options. In 2013, Zhang et al. published “Tryptophan biosynthesis protects mycobacteria from CD4 T-cell-mediated killing” (3). The central finding from that paper is that *M. tuberculosis* must synthesize its own tryptophan in mammalian hosts with functional CD4<sup>+</sup> T cells to cause disease. While these findings were important on their own, the greater impact of this paper stems from the global approaches that provided a path to understanding distinct mechanisms that control tuberculosis (TB) disease at the host-pathogen interface.

To identify the *M. tuberculosis* genes that are required only in the presence of CD4<sup>+</sup> T cell-mediated immunity, Zhang et al. used a forward genetic approach. A pooled transposon library was used in *M. tuberculosis* to infect wild-type C57bl6 mice and mice deficient in CD4<sup>+</sup> T cell responses (major histocompatibility complex class II deficiency [MHCII<sup>-/-</sup>]). *M. tuberculosis* genes that were differentially required in these two strains were identified by transposon insertion sequencing (Tn-seq). By comparing the genes required in mice with functional CD4<sup>+</sup> T cells with results of *in vitro* studies examining predicted stress conditions, the authors found that the *M. tuberculosis* tryptophan biosynthesis pathway is conditionally essential in mice with functional CD4<sup>+</sup> T cells. Given that many intracellular pathogens are tryptophan auxotrophs, it was well known that CD4<sup>+</sup> T cell-derived gamma interferon (IFN- $\gamma$ ) restricts tryptophan availability by inducing the tryptophan-catabolizing enzyme indolamine 2,3-dioxygenase (IDO) (4, 5). Using a combination of *in vivo* and *ex vivo* experiments, the authors carefully showed that an *M. tuberculosis* mutant with a mutation in the tryptophan synthesis pathway ( $\Delta$ TrpE) was unable to survive IFN- $\gamma$ -induced IDO expression in a CD4<sup>+</sup> T cell-dependent manner in both mouse and human cells. This effect could be reversed by increasing the exogenous tryptophan concentration, showing that *M. tuberculosis* must synthesize tryptophan *de novo* in hosts with a functional immune system. To test whether inhibition of *M. tuberculosis* tryptophan synthesis is a viable therapeutic strategy, the authors identified small molecules inhibitors. They found the anthranilate analog 6-FABA synergistically killed *M. tuberculosis* with IFN- $\gamma$ /tumor necrosis factor alpha (TNF- $\alpha$ ) stimulation and reduced *M. tuberculosis* growth *in vivo*. Thus, by defining

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mechanisms that allow *M. tuberculosis* survival during active host immune responses, the authors provided a proof of the principle that targeting immune evasion genes is a realistic therapeutic possibility.

The underlying concepts in the study by Zhang et al. reminded me of a review by Persson and Vance (6) that I read early in my graduate career. In that review, the authors discussed the advantages of combining bacterial and host genetics, or “genetics-squared,” to examine host-pathogen interactions. I was struck by the argument that genetic approaches can be used to tease apart tightly linked interactions between the pathogen and the host, and I was inspired to use this approach in my own research. At the time, I was studying *Chlamydia trachomatis* with a limited genetic toolbox that prevented me from directly using genetic-squared approaches; constraints in genetic editing in *Chlamydia* meant that projects requiring specific mutants became dead ends. For my postdoctoral work, I wanted a genetically tractable pathogen that would allow me to combine both host and bacterial genetic approaches. As I was completing my graduate training in 2013, Zhang et al. published their study, and it profoundly influenced the questions that I would pursue as a postdoctoral fellow and beyond. When I read the paper by Zhang et al., I saw it as a guide for dissecting the genomes of pathogens in immune-defined hosts. Using a library of *M. tuberculosis* mutants as reporters of the environment that bacteria encounter under distinct immune stress conditions or in immune-deficient animals was a powerful approach. While several studies had determined these types of host-pathogen genetic interactions on a candidate basis, the speed and scale at which Zhang et al. completed these studies improved the ability to identify important genes in an unbiased manner in this field (7–10). Additionally, the premise and conclusion of their study were simple and yet profound: the mycobacterial genes that drive immune evasion could be therapeutic targets that, when blocked, could improve *M. tuberculosis* control. That study inspired me to transition to *M. tuberculosis* research for my postdoctoral training. It became clear to me that there was a lack of understanding of how host protective pathways mechanistically function to control *M. tuberculosis* growth and survival (1). I performed bacterial forward genetic screens under a broad range of immune conditions to begin to dissect how *M. tuberculosis* withstands so many distinct host immune mechanisms. For example, using genome-wide bacterial genetic approaches, we examined how bacterial requirements change in distinct immune knockout animals, such as *Nos2*<sup>-/-</sup> animals, that are unable to make nitric oxide (11). We found that hyperinflammation in *Nos2* animals creates a growth-permissive environment, relieving the need for many stress response and nutrient acquisition genes. Several other groups have published similar approaches in different organisms, highlighting how generalizable these host-pathogen genetic interaction studies are and the impact that Zhang et al. had on the field (12–14).

Over the coming years, it is likely that we will continue to see many studies that combine host and pathogen genetics to understand the mechanisms of infectious disease. Not only will forward genetic bacterial screens be performed in defined knockout hosts, but there will likely be an increase in forward host genetic screens using defined bacterial mutants. This strategy was recently published in a tour de force study by Xu et al. that showed the power of host genetic CRISPR approaches to examine the host pathways that drive distinct stress conditions during infection (15). To date, most studies have used only a genome-wide bacterial library and a defined set of host mutants. However, as dual host and pathogen transcriptome sequencing (RNA-seq) is now widespread, it is not difficult to imagine dual host and pathogen genome-wide screening as a real possibility in the near future (16, 17). This technological advance would further the impact of the study by Zhang et al. and would take the genetics-squared approach to the next level by allowing a deeper understanding of how pathogens manipulate immunocompetent hosts and the development of new strategies to treat these infections.

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## REFERENCES

- Nunes-Alves C, Booty MG, Carpenter SM, Jayaraman P, Rothchild AC, Behar SM. 2014. In search of a new paradigm for protective immunity to TB. *Nat Rev Microbiol* 12:289–299. <https://doi.org/10.1038/nrmicro3230>.
- Sia JK, Georgjeva M, Rengarajan J. 2015. Innate immune defenses in human tuberculosis: an overview of the interactions between Mycobacterium tuberculosis and innate immune cells. *J Immunol Res* 2015: 747543. <https://doi.org/10.1155/2015/747543>.
- Zhang YJ, Reddy MC, Ioerger TR, Rothchild AC, Dartois V, Schuster BM, Trauner A, Wallis D, Galaviz S, Huttenhower C, Sacchettini JC, Behar SM, Rubin EJ. 2013. Tryptophan biosynthesis protects mycobacteria from CD4 T-cell-mediated killing. *Cell* 155:1296–1308. <https://doi.org/10.1016/j.cell.2013.10.045>.
- Beatty WL, Belanger TA, Desai AA, Morrison RP, Byrne GI. 1994. Tryptophan depletion as a mechanism of gamma interferon-mediated chlamydial persistence. *Infect Immun* 62:3705–3711. <https://doi.org/10.1128/IAI.62.9.3705-3711.1994>.
- Schmidt SV, Schultze JL. 2014. New insights into IDO biology in bacterial and viral infections. *Front Immunol* 5:384. <https://doi.org/10.3389/fimmu.2014.00384>.
- Persson J, Vance RE. 2007. Genetics-squared: combining host and pathogen genetics in the analysis of innate immunity and bacterial virulence. *Immunogenetics* 59:761–778. <https://doi.org/10.1007/s00251-007-0248-0>.
- Ng VH, Cox JS, Sousa AO, MacMicking JD, McKinney JD. 2004. Role of KatG catalase-peroxidase in mycobacterial pathogenesis: countering the phagocyte oxidative burst. *Mol Microbiol* 52:1291–1302. <https://doi.org/10.1111/j.1365-2958.2004.04078.x>.
- Koster S, Upadhyay S, Chandra P, Papavinasasundaram K, Yang G, Hassan A, Grigsby SJ, Mittal E, Park HS, Jones V, Hsu FF, Jackson M, Sassetti CM, Philips JA. 2017. Mycobacterium tuberculosis is protected from NADPH oxidase and LC3-associated phagocytosis by the LCP protein CpsA. *Proc Natl Acad Sci U S A* 114:E8711–E8720. <https://doi.org/10.1073/pnas.1707792114>.
- Darwin KH, Ehrt S, Gutierrez-Ramos JC, Weich N, Nathan CF. 2003. The proteasome of Mycobacterium tuberculosis is required for resistance to nitric oxide. *Science* 302:1963–1966. <https://doi.org/10.1126/science.1091176>.
- Lightfield KL, Persson J, Brubaker SW, Witte CE, von Moltke J, Dunipace EA, Henry T, Sun YH, Cado D, Dietrich WF, Monack DM, Tsolis RM, Vance RE. 2008. Critical function for Naip5 in inflammasome activation by a conserved carboxy-terminal domain of flagellin. *Nat Immunol* 9:1171–1178. <https://doi.org/10.1038/ni.1646>.
- Mishra BB, Lovewell RR, Olive AJ, Zhang G, Wang W, Eugenin E, Smith CM, Phuah JY, Long JE, Dubuke ML, Palace SG, Goguen JD, Baker RE, Nambi S, Mishra R, Booty MG, Baer CE, Shaffer SA, Dartois V, McCormick BA, Chen X, Sassetti CM. 2017. Nitric oxide prevents a pathogen-permissive granulocytic inflammation during tuberculosis. *Nat Microbiol* 2:17072. <https://doi.org/10.1038/nmicrobiol.2017.72>.
- Shames SR, Liu L, Havey JC, Schofield WB, Goodman AL, Roy CR. 2017. Multiple Legionella pneumophila effector virulence phenotypes revealed through high-throughput analysis of targeted mutant libraries. *Proc Natl Acad Sci U S A* 114:E10446–E10454. <https://doi.org/10.1073/pnas.1708553114>.
- Thibault D, Jensen PA, Wood S, Qabar C, Clark S, Shainheit MG, Isberg RR, van Opijnen T. 2019. Droplet Tn-Seq combines microfluidics with Tn-Seq for identifying complex single-cell phenotypes. *Nat Commun* 10:5729. <https://doi.org/10.1038/s41467-019-13719-9>.
- Goodman AL, McNulty NP, Zhao Y, Leip D, Mitra RD, Lozupone CA, Knight R, Gordon JL. 2009. Identifying genetic determinants needed to establish a human gut symbiont in its habitat. *Cell Host Microbe* 6:279–289. <https://doi.org/10.1016/j.chom.2009.08.003>.
- Xu Y, Zhou P, Cheng S, Lu Q, Nowak K, Hopp AK, Li L, Shi X, Zhou Z, Gao W, Li D, He H, Liu X, Ding J, Hottiger MO, Shao F. 2019. A bacterial effector reveals the V-ATPase-ATG16L1 axis that initiates xenophagy. *Cell* 178:552–566.e20. <https://doi.org/10.1016/j.cell.2019.06.007>.
- Pisu D, Huang L, Grenier JK, Russell DG. 2020. Dual RNA-Seq of Mtb-infected macrophages in vivo reveals ontologically distinct host-pathogen interactions. *Cell Rep* 30:335–350.e4. <https://doi.org/10.1016/j.celrep.2019.12.033>.
- Montoya DJ, Andrade P, Silva BJA, Teles RMB, Ma F, Bryson B, Sadanand S, Noel T, Lu J, Sarno E, Arnvig KB, Young D, Lahiri R, Williams DL, Fortune S, Bloom BR, Pellegrini M, Modlin RL. 2019. Dual RNA-Seq of human leprosy lesions identifies bacterial determinants linked to host immune response. *Cell Rep* 26:3574–3585.e3. <https://doi.org/10.1016/j.celrep.2019.02.109>.