BIOGRAPHICAL SKETCH

NAME: Stacy, Apollo

eRA COMMONS USER NAME: apollostacy

POSITION TITLE: Assistant Staff, Lerner Research Institute, Cleveland Clinic

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Washington University in St. Louis (St. Louis, MO)	B.A.	05/2010	Biology and Earth & Planetary Sciences
The University of Texas at Austin (Austin, TX)	Ph.D.	03/2017	Oral microbiology
National Institutes of Health (Bethesda, MD)	Postdoc	10/2022	Gut microbiome

A. Personal Statement

The diverse microbes that colonize host barrier tissues assemble into heterogeneous communities, where they continuously take part in inter-bacterial interactions, such as the exchange of metabolites. These interactions are highly complex, and they have varying consequences for host fitness. Thus, the focus of my career has been to decipher these interactions, particularly those mediated by microbe and host-derived metabolites, along with their impact on host susceptibility to pathogens. During my PhD, I employed a two-species community, comprising a human oral pathogen and commensal, as a model to dissect oral-microbiota interactions that exacerbate the prevalent inflammatory disease periodontitis (destruction of the tissues that support the teeth). Through my PhD, I gained expertise in bacterial genetics, physiology, and high-throughput approaches for assessing pathogen biology, including Tn-seq (transposon mutant fitness profiling). For my postdoc, I sought to explore more complex model communities and the role of the host in shaping inter-bacterial interactions. Because a hallmark of host adaptive immunity is its ability to acquire memory of past infections, I hypothesized that such encounters can similarly "train" the microbiota's resistance to pathogens. Supporting my hypothesis, I discovered that long after recovering from an enteric pathogen challenge, the gut microbiota can exhibit heightened resistance to subsequent infections. The underlying basis of this microbiota "memory" is host imprinting of commensal metabolism through sustained deployment of a defined metabolite (taurine). This metabolite potentiates the gut microbiota's ability to restrict a pathogen-specific growth strategy, and notably, it exhibits therapeutic potential, as I found that it can be co-opted into a dietary intervention to combat enteric pathogens. Through my postdoc, I expanded my experimental toolkit by gaining expertise in high-throughput approaches for assessing the microbiota, including 16S profiling, metagenomics, and metabolomics. Currently, I am transitioning to establish my independent research lab at Cleveland Clinic, where I will continue to explore how host-derived metabolites shape the ecological balance between commensals and pathogens, particularly in the context of inflammatory diseases and malignancies that afflict/stem from the oral cavity.

Research products as a postdoctoral fellow:

- Stacy A and Belkaid Y (2019). Microbial guardians of skin health. Science. 363(6424):227-228. PMID: 30655428.
- Stacy A, Andrade-Oliveira V, McCulloch JA, Hild B, Oh JH, Perez-Chaparro PJ, Sim CK, Lim AI, Link VM, Enamorado M, Trinchieri G, Segre JA, Rehermann B, Belkaid Y (2021). Infection trains the host for microbiota-enhanced resistance to pathogens. Cell. 184(3):615-627.e17. PMID: 33453153.
- Lim AI, McFadden T, Link VM, Han SJ, Karlsson RM, Stacy A, Farley TK, Harrison OJ, Shih HY, Cameron HA, Belkaid Y. Maternal infection promotes offspring tissue-specific immune fitness (2021). Science. 373(6558):eabf3002. PMID: 34446580.
- Sim CK, Kashaf SS, Stacy A, Proctor DM, Almeida A, Bouladoux N, Chen M, NISC Comparative Sequencing Program, Finn RD, Belkaid Y, Conlan S, Segre JA (2022). A mouse model of intestinal occult colonization

demonstrating antibiotic-induced outgrowth of carbapenem-resistant *Enterobacteriaceae*. Microbiome. 10(1):43. PMID: 35272717

- Stacy A (2022). Training the microbiota to combat infections. Accepted at Science.

B. Positions and Honors

Positions and Employment

2007, 2008	Summer undergraduate researcher, PI: Elizabeth Hood, Arkansas State University
2008–2010	Undergraduate researcher, PI: Brian Faddis, Washington University in St. Louis
2010–2017	Graduate student, PI: Marvin Whiteley, University of Texas at Austin (UT Austin)
2012	Teaching assistant, Introductory Medical Microbiology & Immunology (BIO 326M), UT Austin
2013	Teaching assistant, Antibiotics: Discovery and Function (FRI), UT Austin
2017–2022	Postdoctoral fellow, PI: Yasmine Belkaid, National Institutes of Health (NIH), NIAID
2018, 2021	Guest lecturer, Cellular Immunology: Principles and Methods (BioTech 4), NIH
2022–now	Assistant Staff, Department of Cardiovascular & Metabolic Sciences, Lerner Research Institute, Cleveland Clinic

Honors (as postdoctoral fellow)

2022	Finalist for NOSTER *Science* Microbiome Prize

Travel Awards

2019, 2021	Fellows Award for Research Excellence, NIH
2020	Peggy Cotter Travel Award, American Society for Microbiology (ASM) DC Branch

Oral Presentation Awards

2020	Best Poster (Short Talk) Presentation, NIAID 14th Annual Fellows Workshop, NIH (virtual)
2021	Scholarship, Keystone eSymposium on Harnessing the Microbiome for Disease Prevention and Therapy (virtual)

Oral Presentations (invited or selected from among submitted abstracts)

2019	ASM Microbe, San Francisco, CA
2019	NIAID 13th Annual Fellows Workshop, NIH
2020	Gordon Research Seminar on Microbial Toxins and Pathogenicity (cancelled)
2021	Harvard Chan Center for the Microbiome in Public Health Symposium (virtual)
2021	World Microbe Forum (virtual)
2021	NIGMS Postdoctoral Research Associate Training (PRAT) Symposium on “Diversity, Equity, and Scientific Excellence,” NIH
2021	NIH/FDA Immunology Interest Group Workshop (virtual)
2022	Office of Dietary Supplements Scholars Symposium, NIH
2022	NIDCR Early Career Scientist Seminar Series, NIH
2022	4th International Conference on *Porphyromonas gingivalis* and Related Species in Oral and Systemic Diseases, Louisville, KY
2022	Lambda Lunch, NIH

Professional Activities

2010–now	Member, American Society for Microbiology
2014	Microbial Diversity summer course, Marine Biological Laboratory, Woods Hole, MA
2018–2021	Co-organizer, Microbiome Journal Club (comprising >10 intramural labs), NIH
2019–2020	Elected member, Immunology Interest Group (IIG) steering committee, NIH
2020	RIKEN-Tsinghua International Summer Program, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan (canceled)
2020	Co-organizer, Inaugural IIG Fellows Symposium, NIH

C. Contributions to Science

I played a major role in all 5 of the contributions described below. I made the first 4 contributions as a graduate student and the fifth contribution as a postdoctoral fellow. The central importance of each contribution is underlined.

1. Pathogen fight-and-flight governs polymicrobial synergy and biogeography (graduate student)
As a model to dissect polymicrobial interactions, the lab of my PhD mentor, Marvin Whiteley, studies a two-species community comprising an oral pathogen (*Aggregatibacter actinomycetemcomitans*, *Aa*) and an oral commensal (*Streptococcus gordonii*, *Sg*). Previous work showed that *Aa* displays "synergy" with *Sg* in abscesses (defined as greater numbers in co-infection than mono-infection) and that this synergy hinges upon *Aa*'s ability to cross-feed on lactate, a byproduct of *Sg* fermentation. However, *Sg* also releases high amounts of peroxide, a potent antimicrobial, so it was unclear how *Aa* could benefit from lactate when simultaneously faced with peroxide. While *Aa* expresses a catalase (KatA) that can degrade peroxide, I was intrigued by transcriptomic data showing that oxidative stress also induces the *Aa* biofilm-degrading enzyme Dispersin B (DspB). Based on these data, I hypothesized that peroxide triggers *Aa* biofilm dispersal, thus mitigating stress induced by *Sg*-derived peroxide. Supporting this hypothesis, I showed that DspB is controlled by the peroxide-sensitive regulator OxyR, is induced by *Sg*-derived peroxide, and is required for peroxide-induced biofilm dispersal *in vitro*. In collaboration with Kendra Rumbaugh's group, I then showed that DspB and KatA are required for *Aa* to display synergy with *Sg* during abscess co-infection. To assess how DspB contributes to *Aa* biofilm dispersal *in vivo*, Kendra's group collected confocal images of co-infected abscesses. By analyzing the "biogeography" (spatial organization) of these images, I revealed that *Aa* spaces itself 5-10 μm from *Sg* but, when lacking DspB, is trapped <5 μm from *Sg*, where it likely faces greater oxidative stress and can no longer display synergy. Based on these results, I developed a "fight-and-flight" model to describe *Aa*-*Sg* synergy, in which *Aa* both fights/degrades (via KatA) and flees/disperses (via DspB) from *Sg*-derived peroxide in order to gain a benefit from cross-feeding on *Sg*-derived lactate. This work was the first to demonstrate that μm-scale biogeography (spatial organization) can impact the virulence of polymicrobial infections. In collaboration with Sam Brown's group, I synthesized my findings with previous literature in a review about the spatial organization of infections.

- Stacy A, Everett J, Jorth P, Trivedi U, Rumbaugh KP, Whiteley M (2014). Bacterial fight-and-flight responses enhance virulence in a polymicrobial infection. Proceedings of the National Academy of Sciences. 111(21):7819-24. PMCID: PMC4040543.
  - Highlighted by Nature and Nature Reviews Microbiology
- Stacy A, McNally L, Darch SE, Brown SP, Whiteley M (2016). The biogeography of polymicrobial infection. Nature Reviews Microbiology. 14(2):93-105. PMCID: PMC5116812.

2. Pathogen cross-respiration governs polymicrobial synergy (graduate student)
The oral pathogen *A. actinomycetemcomitans* (*Aa*) displays synergy (enhanced virulence) with the oral commensal *S. gordonii* (*Sg*) in abscesses, and this synergy hinges upon *Aa*'s ability to cross-feed on *Sg*-derived lactate. Because lactate is non-fermentable, *Aa* can only utilize lactate via respiration, requiring an electron acceptor such as oxygen. However, abscesses are commonly assumed to be anaerobic, raising the question of how *Aa* can benefit from lactate in such an infection environment. To tackle this question, I took a "biosensor" approach. Since measuring the potentially μm-scale gradients of oxygen in the abscess would be challenging, I reasoned that I could instead measure the fitness of oxygen-sensitive *Aa* mutants as a proxy. To identify such mutants both rapidly and comprehensively, I generated a large pool of *Aa* transposon mutants and developed a protocol for transposon-seq (Tn-seq), or high-throughput transposon mutant fitness profiling. This protocol isolates and prepares the DNA flanking transposon insertions for deep-sequencing, and with it, I could profile the relative abundance of individual *Aa* transposon mutants within my pool of mutants. I passaged this pool *in vitro* through anaerobic and aerobic conditions and *in vivo* through the abscess as a mono- or co-infection with *Sg*. By carefully comparing these 4 conditions, I inferred that the abscess contains a mixture of oxygen micro-environments and that *Sg* increases oxygen's overall availability to *Aa*. Additionally, I found that the aeration of low-oxygen infection environments (abscesses) by commensals (*Sg*) allows pathogens (*Aa*) to switch from fermentation to respiration, demonstrating that commensals can support the ability of pathogens to respire with non-fermentable commensal-derived substrates. Because I discovered that *Sg* not only provides carbon (lactate) but also a substrate (oxygen) required for *Aa* to cross-feed on this carbon via respiration, I named this phenomenon "cross-respiration." To extend these findings, I collected a panel of 25 oral (sympatric) and non-oral (allopatric) microbes and individually co-infected these microbes with the *Aa* mutant pool in the abscess. In collaboration with Gina Lewin, Tn-seq on all 25 co-infections revealed that cross-respiration is a common feature of polymicrobial synergy. These results are important because they indicate that cross-respiration is a promising therapeutic target.

- Stacy A, Fleming D, Lamont RJ, Rumbaugh KP, Whiteley M (2016). A commensal bacterium promotes virulence of an opportunistic pathogen via cross-respiration. mBio. 7(3):e00782-16. PMCID: PMC4916382.
  - Highlighted by mBio (Editor's pick)
- Lewin GR, Stacy A, Michie KL, Lamont RJ, Whiteley M (2019). Large-scale identification of pathogen essential genes during coinfection with sympatric and allopatric microbes. Proceedings of the National Academy of Sciences. 116(39):19685-19694. PMCID: PMC6765283.
  - Highlighted by This Week in Microbiology podcast

3. Polymicrobial infection composition governs pathogen iron availability (graduate student)
Because iron is an essential micro-nutrient for microbial pathogenesis, it is actively sequestered by the host, resulting in nutritional immunity. Numerous studies have described in depth the variety of mechanisms by which pathogens overcome nutritional immunity. These studies, however, often assume that the host restricts iron to constitutively low levels, when in fact the availability of iron can fluctuate as a result of environmental factors such as diet. Because nearly all microbes require iron, I hypothesized that commensal microbes influence iron's availability to pathogens. To test this hypothesis, I again took a "biosensor" approach. I performed RNA-seq on *A. actinomycetemcomitans* (*Aa*) *in vitro* as biofilms under high and low iron conditions and, in collaboration with Peter Jorth, *in vivo* in abscesses as mono- or co-infections with *S. gordonii* (*Sg*). In collaboration with an undergraduate mentee, Nader Abraham, I performed ChIP-seq on the iron-responsive regulator Fur both *in vitro* and *in vivo*. By comparing the undefined *in vivo* conditions with the defined *in vitro* conditions, I inefrred that *Aa* iron starvation in the abscess is heightened by co-infection with *Sg*. To extend these findings, I compared my RNA-seq data with Peter Jorth's published meta-transcriptomic data of healthy and diseased human dental plaque, and discovered that periodontal disease also exacerbates *Aa* iron starvation. Together, these findings showed that a defined commensal and the quality of polymicrobial communities (healthy vs. diseased) can shape a pathogen's access to the essential nutrient iron.

- Stacy A, Abraham N, Jorth P, Whiteley M (2016). Microbial community composition impacts pathogen iron availability during polymicrobial infection. PLOS Pathogens. 12(12):e1006084. PMCID: PMC5156373.

4. Ordered transposon mutant library for *Aggregatibacter actinomycetemcomitans* (graduate student)
Despite the medical significance of *Aa*'s association with periodontitis in adolescents, many genetic tools are still lacking for this pathogen. Thus, an undergraduate mentee, Ajay Narayanan, and I collaborated on the Herculean task of constructing an ordered *Aa* transposon mutant library, for which we identified the location (exact well and microtiter plate) of >1500 unique mutants. Of note, we drastically cut the labor required for this task by executing a strategic sequencing-based approach (Cartesian pooling-coordinate sequencing). The advantage of our ordered library of >1500 unique transposon mutants, which we made publicly available to the *Aa* research community, is that it allows ready access to mutant(s) in nearly any *Aa* gene of interest, preventing unwanted delays to projects.

- Narayanan AM, Ramsey MM, Stacy A*, Whiteley M* (2017). Defining genetic fitness determinants and creating genomic resources for an oral pathogen. Applied and Environmental Microbiology. 83(14):e00797-17. PMCID: PMC5494627. *Co-corresponding author
  - Highlighted by Applied and Environmental Microbiology (Editor's pick)

5. Infection trains the microbiota for enhanced resistance to pathogens (postdoctoral fellow)
One of the most important functions of the gut microbiota is to provide "colonization resistance," or protection against colonization by pathogens. Much of the research on colonization resistance uses mice that are specific-pathogen-free. However, this approach fails to capture the complex infection history of hosts in their natural environments, which led me to ask: how do past infections influence the microbiota's response to future infections? Due to the prevalence of infections throughout evolution, I hypothesized that hosts have adapted to exploit infections as opportunities to train and optimize their microbiota, analogous to how the microbiota in turn educates the immune system and establishes immunological memory. To investigate this question, I employed two mouse models: (1) mice with a defined previous infection by an attenuated strain of *Yersinia pseudotuberculosis* and, in collaboration with Barbara Rehermann's (NIDDK) group, (2) germ-free mice that received the gut microbiota of wild mice, since these mice undoubtedly encounter more infections than lab mice. I discovered that infection-trained gut microbiota indeed confer enhanced resistance to subsequent gut infection by *Klebsiella pneumoniae*. In collaboration with Giorgio Trinchieri's (NCI) group, I then performed metagenomics using the two mouse models, revealing the striking joint enrichment of functions related to the utilization of

taurine. As predicted by metagenomics, I then showed, via metabolomics, that taurine is indeed elevated in the gut following infection, likely as a result of heightened host synthesis of taurine-conjugated bile acids. Next, I showed that exogenous taurine alone, without prior infection, is sufficient to enhance colonization resistance. Furthermore, metagenomics revealed that, as in infection-trained mice, exogenous taurine enriches for the production of sulfide, the final byproduct of taurine metabolism. To dissect how taurine-derived sulfide potentially controls *K. pneumoniae* colonization, I resorted to Tn-seq, revealing that sulfide restricts *K. pneumoniae*'s ability to respire, thus blocking its access to the non-fermentable substrate 1,2-propanediol. To further support these findings, I showed that sequestering sulfide via bismuth not only greatly impairs colonization resistance to *K. pneumoniae* but also unleashes other respirers in the gut microbiota, suggesting that the gut microbiota's production of sulfide is an ecological keystone function. Together, my findings support the following model: infection triggers the host to nourish the microbiota with taurine, the microbiota converts taurine to sulfide, and sulfide restricts the respiration of invading pathogens. Notably, these findings support the concept that the microbiota, like the immune system, can be trained to acquire a memory-like state of enhanced resistance.

- Stacy A, Andrade-Oliveira V, McCulloch JA, Hild B, Oh JH, Perez-Chaparro PJ, Sim CK, Lim AI, Link VM, Enamorado M, Trinchieri G, Segre JA, Rehermann B, Belkaid Y (2021). Infection trains the host for microbiota-enhanced resistance to pathogens. Cell. 184(3):615-627.e17. PMID: 33453153.
  - Highlighted by Nature, Nature Reviews Microbiology, Cell, Immunity, Trends in Endocrinology & Metabolism, Cell Research
- Stacy A (2022). Training the microbiota to combat infections. Accepted at Science.

Complete list of publications: https://pubmed.ncbi.nlm.nih.gov/?term=apollo+stacy

D. Additional Information: Research Support and/or Scholastic Performance

Current Research Support

NIH K99DE031372 $463,975 09/01/2021 - 08/31/2025
Title: Role of sulfide in oral microbiota-host interactions that promote periodontitis
Goals: (1) Assess the role of *Filifactor alocis*-derived sulfide in promoting *A. actinomycetemcomitans* (*Aa*) anaerobic respiration and (2) assess the role of *F. alocis*-derived sulfide in promoting immuno-pathology.

Cleveland Clinic startup package $1,811,000 10/2022 - 10/2027

Completed Research Support

NIH F31DE024931 09/12/2014 - 03/19/2017
Title: Identifying disease mechanisms of a periodontal pathogen
Goals: (1) Determine how the *Aa* biofilm-dissolving enzyme Dispersin B serves as a strategy to avoid host iron restriction and (2) determine the *Aa* transcriptome and genome-wide fitness determinants during co-infection with the commensal *Streptococcus gordonii*.

NIH FI2GM128736 09/01/2018 - 08/31/2021
Title: Impact of metals on the skin microbiome: consequences for antibiotic resistance and inflammation
Goals: (1) Determine how aluminum, the active ingredient in antiperspirants, selects for antibiotic resistance in skin commensals, (2) discover metabolites for treating wounds infected with aluminum-resistant skin commensals, and (3) discover metabolites for treating aluminum-induced dysbiosis and inflammation.

NIH Office of Dietary Supplements 10/01/2020 - 9/30/2021
Research Scholars Program
Title: Impact of taurine on the gut microbiota in health and disease
Goals: (1) Determine the impact of taurine and taurine-derived sulfide on the gut microbiota and (2) determine the impact of taurine and taurine-derived sulfide on a gut pathogen.