HHS Public Access

Author manuscript

Cell Host Microbe. Author manuscript; available in PMC 2016 November 11.

Published in final edited form as:

Cell Host Microbe. 2015 November 11; 18(5): 613–620. doi:10.1016/j.chom.2015.10.009.

Individual members of the microbiota disproportionately modulate host innate immune responses

Annah S Rolig[1], **Raghuveer Parthasarathy**[2], **Adam R Burns**[3], **Brendan JM Bohannan**[3], and **Karen Guillemin**[1,*]

[1]Institute of Molecular Biology, 1370 Franklin Blvd, University of Oregon, Eugene, OR, USA 97403

[2]Department of Physics, 1274 University of Oregon, Eugene, OR, USA 97403

[3]Institute of Ecology and Evolution, 5289 University of Oregon, Eugene, OR, USA 97403

Summary

Predicting host health status based on microbial community structure is a major goal of microbiome research. An implicit assumption of microbiome profiling for diagnostic purposes is that the proportional representation of different taxa determine host phenotypes. To test this assumption, we colonized gnotobiotic zebrafish with zebrafish-derived bacterial isolates and measured bacterial abundance and host neutrophil responses. Surprisingly, combinations of bacteria elicited immune responses that do not reflect the numerically dominant species. These data are consistent with a quantitative model in which the host responses to commensal species are additive, but where various species have different per capita immunostimulatory effects. For example, one species has a high per capita immunosuppression that is mediated through a potent secreted factor. We conclude that the proportional representation of bacteria in a community does not necessarily predict its functional capacities; however, characterizing specific properties of individual species offers predictive insights into multi-species community function.

Introduction

Animals and their resident microbial communities, or microbiota, are a complex ecosystem. These microbes derive nutrients from the host environment, and in turn, they influence normal animal development and health. The gastrointestinal microbiota are critical for nutrient acquisition and immune system development (Bäckhed et al., 2005; Hooper et al., 2012). Metagenomic profiling of gut microbiota has identified deviations from taxonomic compositions associated with health in diseases such as obesity (Turnbaugh et al., 2009),

*Corresponding author: Phone: 541-346-5360, guillemin@molbio.uoregon.edu.

Author contributions

Conceptualization, ASR and KG; Methodology, ASR and RP; Formal analysis, ASR, RP, and ARB; Investigation, ASR; Writing – Original Draft, ASR and RP; Writing – Review & Editing, ASR, RP, ARB, BJMB, and KG; Funding Acquisition, ASR and KG; Resources, KG; Supervision, KG and BJMB.

diabetes (Wen et al., 2008), and inflammatory bowel diseases (IBD)(Frank et al., 2007). An implicit assumption in these compositional analyses is that the relative abundances of different taxa can predict pathology; however, application of this assumption to clinical data does not uncover consistent trends. For example, both an increased (Turnbaugh et al., 2009) and decreased ratio (Jumpertz et al., 2011) of Bacteroidetes to Firmicutes have been associated with obesity. Additionally, a meta-analysis of human obesity-associated microbiota concluded that small shifts in many taxa, rather than large differences in a few taxa, are more likely to predict obesity (Walters et al., 2014). Thus, the extent to which microbiota composition can be used to predict community function and human health status remains an open question.

The complexity and variability of vertebrate-associated microbiota presents substantial challenges to unraveling their functional potential. For example, DNA sequence-based surveys of microbiota cannot distinguish between active and inactive or resident and transient members. Another limitation of such surveys is that they only provide information on the proportional representation of taxa but not their per capita contributions to community functions, such as the capacity to induce an inflammatory response. These limitations emphasize the need for simplified, defined model systems to connect the composition of resident bacterial communities with their emergent properties. We created a tractable system to study the impact of microbiota composition on the intestinal innate immune response using the zebrafish, *Danio rerio*. The zebrafish is an excellent model to examine microbial community function because hundreds of zebrafish can be easily derived and maintained in a germ-free (GF) or gnotobiotic state with defined microbial isolates (Milligan-Myhre et al., 2011). The zebrafish intestinal microbial community is well-characterized; a large number of intestinal microbes that span the phylogenetic diversity observed in the zebrafish microbiota can be maintained in culture and have had their genomes sequenced (Stephens et al., 2015). Furthermore, zebrafish transgenesis and optical transparency allows for high resolution monitoring of host and microbial cells *in vivo* (Jemielita et al., 2014). We exploited these properties to develop an assay in which we monitor both the composition of the bacterial community and the innate immune response in an individual fish, using GFP-expressing neutrophils as a metric of the host response. Neutrophils are a primary component of the initial inflammatory response and critical for host defense (Harvie and Huttenlocher, 2015). Neutrophil homeostasis is established and maintained by the microbiota, as GF larvae have reduced intestinal (Bates et al., 2007) and systemic neutrophils and reduced neutrophil responses to injury (Kanther et al., 2014). Thus neutrophil dynamics are a sensitive measure of host responses to intestinal microbiota.

Here we use our gnotobiotic zebrafish model to measure the host neutrophil response to individual microbiota constituents and small communities assembled from these members. We show that the per capita immunostimulatory effect of individual species within a community varies widely, such that minor members can exert dominant effects. A simple mathematical model based on additive responses to individual species describes the neutrophil response to these communities by accounting for the per capita effect of each species. Our approach demonstrates the feasibility of predicting the function of a microbial community based on its structure, which in the future may be expanded to more complex

systems to improve our understanding of human disease-associated microbial communities and our ability to restore them to a healthy state.

Results

Microbial isolates induce unique neutrophil responses

To assay the influence of individual bacterial species on the intestinal innate immune response, we raised GF zebrafish and inoculated their aquatic environment with single bacterial isolates (mono-associations) from our collection of zebrafish intestinal bacteria (Stephens et al., 2015). Bacteria were introduced at 4 days post fertilization (dpf), by which time their intestine had opened, and at 6 dpf we dissected the intestine and assessed neutrophil populations (Fig. 1A) and bacterial colony forming units (CFU) per intestine. All neutrophil responses to the individual strains we tested were within the range observed for GF and conventionalized (CVZ) fish, yet there was a wide range of responses both between and within groups (Fig. 1B). For some strains the variation in neutrophil response correlated with variation in bacterial abundance. For example, for a *Vibrio* species, the number of neutrophils increased with bacterial abundance and was fit by a linear relationship between neutrophil number and log(CFU) (Fig. 1D). The log(CFU) of two species, *Shewanella* (Fig. 1D) and *Acinetobacter* (not shown), were negatively correlated with neutrophil number. A third pattern, characteristic of most isolates, represented by *Aeromonas*, displayed no clear relationship between neutrophil and bacterial abundance (Fig. 1D). We used representatives of each of these three species-specific neutrophil responses to explore whether we could predict the neutrophil response to more complex communities.

Complex dynamics in microbial di-associations influence microbial abundance and neutrophil response

To test whether the relationship between bacterial and neutrophil abundance in mono-association is indicative of their contribution in a complex community to the neutrophil population, we examined every dual species combination (di-association) between *Aeromonas*, *Vibrio*, and *Shewanella*. In a di-association, the two species are added together at the same concentration to the aquatic environment of GF fish at 4 dpf, and the CFU/gut and intestinal neutrophil influx are assayed at 6 dpf. In di-associations between *Vibrio* and either *Shewanella* or *Aeromonas*, *Vibrio* was the numerically dominant member and its abundance was unchanged or increased, respectively, compared to its abundance in a mono-association (Fig. 2A). In the di-association with *Vibrio*, *Aeromonas* was undetectable in 55% of fish and when it did co-colonize with *Vibrio*, it was present at a significantly lower abundance than in a mono-association (Fig. 2B). Notably, in fish colonized with *Aeromonas*, *Vibrio* abundance increased compared to the fish with no detectable *Aeromonas* (Fig. 2A). In terms of relative abundance, *Vibrio* dominated the di-associations with *Aeromonas* (98% ± 1%) and *Shewanella* (89% ± 3%), and *Shewanella* dominated the di-association with *Aeromonas* (97% ± 1%) (Fig. 2C, 2D). The abundance of these species in the water did not change in comparison to mono-associations (Fig. S1A), indicating that the dynamics are host-associated.

In di-associations between *Aeromonas* and either *Shewanella* or *Vibrio*, the neutrophil response reflected the dominant member (Fig. 2D, 2E, S1B, S1C). Notably, in the *Vibrio* and *Aeromonas* di-association, neutrophil influx was higher than predicted given the relative proportion of members and a simple expectation of a sum of neutrophil responses (Fig. 2E, grey bars). However, the expectation that the dominant species determines the neutrophil response failed in the *Vibrio* and *Shewanella* di-association. In this case, *Vibrio* was the dominant species (Fig. 2D), yet intestinal neutrophil influx was significantly reduced compared our expectation (Fig. 2E, grey bars). In fact, neutrophil influx was similar to a *Shewanella* mono-association (Fig. 2E, S1C), which suggests that the minor species *Shewanella* had a disproportionate impact on the neutrophil response.

A model of additive responses to bacterial species can explain intestinal neutrophil responses in di-associations

Because neutrophil responses to *Vibrio* di-associations with *Aeromonas* and *Shewanella* differed from a simple expectation (Fig. 2E), we explored whether we could construct a mathematical model of the neutrophil response to two-member communities, based on knowledge of the responses to individual species (Fig. 1D) and their abundances in di-associations (Fig. 2A–C). To avoid over fitting the data, we constructed a minimal model that parameterizes key aspects of bacterial growth and interactions between bacterial species and neutrophils. We modeled bacterial growth and competition with Lotka-Volterra equations (eq. 1), which apply to a variety of ecological systems including host-associated microbial communities (Fisher and Mehta, 2014; Marino et al., 2013; Stein et al., 2013):

$$\frac{d}{dt}P_i = r_i P_i(1 - (P_i + \gamma_{ij} P_j - b_i N)K_i^{-1}) \quad (1)$$

where P_i, r_i, and K_i denote the population, growth rate, and carrying capacity, respectively, of species *i*, γ_{ij} characterizes the effect of species *j* on the dynamics of species *i*, and b_i defines the effect of the neutrophil population on species *i*. We modeled the neutrophil population (*N*) with linear influx and exit terms and, importantly, an additive contribution from each bacterial species (eq. 2):

$$\frac{d}{dt}N = \alpha_N - k_N N + \Sigma_i \alpha_i(P_i) \quad (2)$$

where α_N and k_N are the influx and exit rate of neutrophils, respectively, and α_i is the effect of species *i* on neutrophil influx. Inspired by the observed form of the mono-association data (Fig. 1D), we modeled α_i as being linearly dependent on the logarithm of bacterial abundances (eq. 3):

$$\alpha_i(P_i) = M_i \log_{10}\left(\frac{P_i}{T_i}\right) \quad (3)$$

where M_i characterizes the slope of the bacteria-neutrophil interaction and T_i is the effective threshold for a positive effect. For *Vibrio*, we constrain $\alpha > 0$ to specify that no population levels suppress neutrophil numbers. We also considered a sigmoidal model of bacteria-neutrophil interactions, which yields similar behaviors (Supplemental methods).

Experiments suppressing the immune response (Fig. 3) and simulations (Supplemental methods) imply that the data can be modeled without incorporating potential influences of neutrophils on bacterial abundance; i.e. b_i can be set to zero, and Equation 1 is independent of N. This and other omitted interactions may exist in more complex communities; however, we aimed to determine whether a minimal model could describe our observed di-association data.

Most parameters (r_i, K_i, α_N/k_N, γ_{ij}, M_{Vibrio}, and T_{Vibrio}) are well constrained by experimental data. If the slope and threshold parameters for the influence of *Shewanella* and *Aeromonas* mono-associations on neutrophil influx were precisely known, all model parameters would be fixed. The scatter in the data (Fig. 1D) prevent this, but we can examine the M_i/T_i parameter space for regions that are consistent with *both* the mono-association data and the observed neutrophil number in di-associations of each of these species with *Vibrio*. For both di-associations with *Vibrio*, we find such overlapping regions in parameter space (Supplemental methods). Thus, our additive model of bacterial/neutrophil interactions is sufficient to describe the observed data. This indicates that relatively few *Shewanella* are required to dominate the immune response; their large per capita effect is parameterized by a combination of large slope M and low threshold T. The success of a simple additive model in predicting the host neutrophil response to a two-member bacterial community suggests that 1) neutrophil feedback on bacterial populations is negligible in the context of normal neutrophil responses to commensals and 2) certain disproportionately impactful species, like *Shewanella*, may use interesting mechanisms to influence neutrophil dynamics in complex, multi-species communities.

An interaction between Vibrio and Aeromonas drives Vibrio growth and neutrophil influx

The model predicted that the higher than expected neutrophil influx in the di-association between *Vibrio* and *Aeromonas* was independent of neutrophil feedback, and likely dependent on an increase in *Vibrio* abundance conferred by the presence of *Aeromonas.* An alternative explanation, inconsistent with our model, would be that increased *Vibrio* abundance occurs as a result of positive feedback from the neutrophil influx elicited by *Aeromonas*, with *Vibrio* behaving like a pathobiont that thrives in an inflamed environment (Mazmanian et al., 2008). To distinguish between these two possibilities we implemented two independent means of immune suppression, prednisolone (Oehlers et al., 2011; a steroid immunosuppressant) and a tumor necrosis factor receptor (*tnfr*) morpholino (Bates et al., 2007; which blocks pro-inflammatory TNFα signaling; Fig. 3A). We found that under conditions of low neutrophil influx, *Vibrio* abundance still increased in the di-association with *Aeromonas* in comparison to the *Vibrio* mono-association (Fig. 3B). Prednisolone did not affect the growth of *Vibrio* or *Aeromonas in vitro* (Fig. S2) or in mono-association (Fig. 3C). These data support our model's prediction that neutrophils do not feedback on bacterial abundance and suggest an interaction between *Vibrio* and *Aeromonas*. The slope of the relationship between the logarithm of *Vibrio* abundance and neutrophil influx was unchanged in the di-association compared to the *Vibrio* mono-association (Fig. 3D), however the intercept is higher, suggesting that either *Aeromonas* contributes to the neutrophil influx or *Vibrio* has an increased per capita effect in the presence of *Aeromonas*.

We further explored the interaction between *Aeromonas* and *Vibrio* by asking whether they influence each other's populations when grown in direct contact or in close vicinity *in vitro.* Compared to a co-culture with differentially marked isogenic strains cross species co-culture promoted the growth of *Vibrio* and inhibited growth of *Aeromonas* in a contact dependent manner (Fig. 3E). These experiments establish that we can recapitulate *in vitro* an inter-species interaction that occurs *in vivo* and alters the potential of the community to induce intestinal neutrophil influx.

Shewanella controls the neutrophil response via a secreted anti-inflammatory factor

As a minor member of the di-association with *Vibrio*, *Shewanella* directed a lower than expected neutrophil response (Fig. 2E) and abolished the relationship between *Vibrio* abundance and neutrophil influx (Fig. 4A). Our model posited that *Shewanella* exerted a large per capita effect on the neutrophil response, which we reasoned could be mediated through a potent secreted product. When we treated *Vibrio* mono-associated fish with 500-ng/ml concentrated *Shewanella* cell-free supernatant (CFS), we observed that a secreted factor (or factors) from *Shewanella* was sufficient to induce a low neutrophil response to *Vibrio* (Fig. 4B), while *Vibrio* abundance remained unaltered (Fig. S3A). Heat killing of *Shewanella*, which inactivates secretion and denatured proteins, eliminated *Shewanella's* effect (Fig. 4B). Interestingly, *Shewanella* CFS did not alter neutrophil influx (Fig. 4C) or abundance (Fig. S3B) in an *Aeromonas* mono-association, which suggests either that *Shewanella's* anti-inflammatory factor specifically inhibits a pro-inflammatory activity of *Vibrio* or that *Aeromonas* inactivates the anti-inflammatory factor.

Finally, we examined the host-microbiota system with the three-member community. The intestines of fish inoculated with equal parts *Aeromonas, Vibrio,* and *Shewanella* were dominated by *Vibrio* (71% ± 7%), with *Shewanella* contributing 28% ± 7%, and *Aeromonas* contributing 1% ± 0.3% (Fig. 4D). Despite the numerical dominance of *Vibrio,* intestinal neutrophil influx was significantly lower than observed in the *Vibrio* and *Aeromonas* di-association (Fig. 4E). Furthermore, *Shewanella* CFS was sufficient to elicit this phenotype when added to a *Vibrio* and *Aeromonas* di-association (Fig. 4E). Thus, in a three-member microbial community, a numerically minor member can determine the neutrophil response to the community through the activity of a potent secreted anti-inflammatory factor (Fig. 4F).

Discussion

Two major challenges of microbiome research are to use compositional data to predict the functions of a complex microbial community, such as its inflammatory potential, and to manipulate community membership to promote a specific function. Here, we describe a simple mathematical model that accounts for both competition between microbes and the immunomodulatory effect of each member on the host and predicts the collective immune response elicited by the composite community as the sum of the effects of each individual member, scaled to its particular per capita effect. Our model demonstrates the feasibility of predicting the function of a microbiota based on its composition when specific properties of the individual species are known. Our modeling approach could be expanded to more

complex systems, such as the mouse or human gastrointestinal tract, where the mono-association data in our model could be replaced with data based on other individual traits, such as pro- or anti-inflammatory properties measured in a cell based assay (Mastropietro et al., 2015). It will be interesting to see whether other functions of complex microbial communities, such as carbohydrate metabolism in mice (Sonnenburg et al., 2006) and nutrient acquisition in flies (Newell and Douglas, 2014) are consistent with additive contributions from species with different per capita effects or whether a quantitative description of these systems will require evoking non-additive interactions. Finally, our model predicts non-monotonic changes in the neutrophil population over time (Supplemental methods), which may be observable with live imaging of host-bacterial dynamics in real time (Jemielita et al., 2014).

From our model we also gained mechanistic insights into bacterial-bacterial and bacterial-host interactions within the system, which is a step toward manipulating a community to secure a desired function. Our modeling and experimental analysis suggested that in our system, bacterial-bacterial interactions play the dominant role in determining community membership, with no evidence for neutrophil feedback on the bacterial populations. We speculate that this would not be true in a pathologically inflamed intestine, where certain members would likely experience growth inhibition and other inflammation-adapted species would thrive (Winter et al., 2010). We observed a strong bacterial-bacterial interaction between *Vibrio* and *Aeromonas* both when co-colonizing the zebrafish intestine and growing in contact *in vitro*. In the intestine, *Vibrio*'s impairment of *Aeromonas* growth correlated with an increase in *Vibrio* abundance, and thus a corresponding increase in the neutrophil recruiting capacity of the community. Given the contact dependent nature of the *in vitro* interaction between *Vibrio* and *Aeromonas*, it is possible that this interaction involves a type VI secretion system (MacIntyre et al., 2010; Stephens et al., 2015). Our ability to replicate the *in vivo* dynamic between *Aeromonas* and *Vibrio in vitro* highlights a strength of our system and allows us to further interrogate the mechanism of interaction between these species.

The microbiota and the host must maintain a homeostatic relationship both to activate neutrophils for responding to injury and infection (Kanther et al., 2014) and to allow the resident microbes to persist. The range of neutrophils required to establish this relationship is represented in CVZ fish, and all examined mono-associations were within this range. Notably, the average neutrophil response to each bacterial isolate was proportional to that species' average abundance (Fig. S3C), consistent with the observation that generic bacterial immunostimulatory molecules, such as lipopolysaccharide, contribute to the regulation of neutrophil influx (Bates et al., 2007). However, different isolates exhibited different relationships between neutrophil number and bacterial load across individual mono-associated fish, suggesting that individual bacteria have specialized mechanisms by which they influence the host neutrophil response.

In both two- and three-member communities *Shewanella* acts as a keystone species (Power et al., 1996) by exerting a disproportionately large effect on the neutrophil population given its low abundance. *Shewanella* strains are used as probiotics in aquaculture (Tapia-Paniagua et al., 2014), suggesting that they retain immunodominance in complex, natural

communities. The human intestinal microbiota contains many low abundance species (Arumugam et al., 2011), and some have a disproportionately large impact on inducing dysbiosis and disease (Hajishengallis et al., 2012) or on promoting health (Sokol et al., 2008). For example, *Faecalibacterium prausnitzii*, whose absence correlates with IBD (Cao et al., 2014; Sokol et al., 2008, 2009), comprises only 4 – 6% of the mucosa-associated microbiota, yet it reduces pro-inflammatory cytokine signaling and colitis severity through a secreted anti-inflammatory factor (Sokol et al., 2008). Similarly, in our system *Shewanella* generates a low neutrophil response via a secreted anti-inflammatory factor. We do not know whether this anti-inflammatory factor acts on the host or on *Vibrio;* however, the abundance of *Vibrio* is slightly, although not significantly, reduced in the presence of *Shewanella* and its CFS. This slight reduction in *Vibrio* may contribute to a reduced neutrophil response, or alternatively it may be the result of a low inflammatory environment elicited by *Shewanella*. Such an environmental alteration is a characteristic of a keystone species. Given the central role keystone species play in ecosystem function, identifying them will be critical for our ability to engineer microbial communities to promote a required function. Here we have identified one such species and identified two measurable properties —a high per capita effect and a negative relationship between abundance and neutrophil response—that may be used to screen for other such species. Identifying critical players with large per capita effects, like *Shewanella*, will advance our ability both to predict community functions and to manage community membership.

Experimental procedures

For additional details, see supplemental materials and methods.

Gnotobiotic zebrafish husbandry

All zebrafish experiments were performed following protocols approved by the University of Oregon Institutional Animal Care and Use Committee. Conventionally-raised wild-type (AB × Tu strain) and Tg(BACmpx:GFP)i114 (referred to as mpx:GFP) (Renshaw et al., 2006) were maintained as described (Westerfield, 1993). Zebrafish embryos were derived GF and associated with bacterial isolates as previously described (Bates et al., 2006). At 6 dpf the mpx:GFP zebrafish were anesthetized in Tricaine (Western Chemical, Inc., Ferndale, WA), mounted in 4% methylcellulose (Fisher, Fair Lawn, NJ), and their intestines were dissected using sterile technique. The number of GFP-positive cells was quantified visually for each fish using a fluorescent microscope (SteREO Discovery.V8, Zeiss).

Microbiology

Bacteria used for inoculations were zebrafish isolates ZOR0001 *(Aeromonas),* ZWU0020 *(Vibrio),* ZOR0012 *(Shewanella),* ZNC0006 *(Variovorax),* ZNC0008 *(Delftia),* ZOR0008 *(Acinetobacter),* ZOR0002 *(Aeromonas sp. 2),* ZWU0006 *(Pseudomonas),* ZOR0011 *(Pleisomonas),* and ZOR0014 *(Enterobacter)* (Stephens et al., 2015). To determine the CFU/intestine, dissected zebrafish intestines were placed in 100-μl sterile EM, homogenized, diluted, and cultured on tryptic soy agar plates (TSA; BD, Sparks MD). For di- and tri-associations, bacterial species were distinguished by colony morphology.

Morpholino injections

Splice-blocking MOs (Gene Tools, Corvallis, OR) were injected into the embryos at the one cell stage. The TR1v1/TR1v2 (1.2 moles and 6 moles, respectively) were used as previously described (Bates et al., 2007).

Prednisolone treatments

The prednisolone solution was prepared and administered as described (Oehlers et al., 2011).

Concentration of CFS

Shewanella was grown over night shaking in TSB. 1 ml of overnight culture was used to inoculate 50-ml TSB, which was kept shaking at 30° C for 2 h. The supernatant was filtered (Corning Inc., Corning NY) and concentrated with a centrifugal device with a 10-kda weight cut off (Pall Life Sciences, Ann Arbor, MI).

In vitro co-culture assay

Vibrio and *Aeromonas* were grown overnight shaking in TSB (BD, Sparks, MD). 5×10^8 bacterial cells of each strain were mixed together and spotted onto filter paper on brain heart infusion media agar plate (BHI, BD, Sparks, MD). A co-culture of an isogenic fluorescently tagged strain with the wild-type counterpart served as controls. To determine contact dependency, the filter paper was placed between the strains (MacIntyre et al., 2010).

Statistics and modeling

Statistical analysis was performed using Prism (Graphpad Software). Statistical significance was defined as $p < 0.05$. Modeling details can be found in Supplemental experimental methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Rose Sockol and UO Zebrafish Facility staff for fish husbandry. We thank Guillemin lab members for insightful discussions and Tiffani Jones for critical reading of the manuscript. Research reported in this publication was supported by the NIH: by the NIGMS under award number P50GM098911, by the NIDDK under award number 1F32DK098884-01A1 (to ASR), and by the NICHHD under award P01HD22486, which provided support for the UO Zebrafish Facility. The content is solely the responsibility of the authors and does not represent the official views of the NIH.

References

Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto J-M, et al. Enterotypes of the human gut microbiome. Nature. 2011:1–7.

Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005; 307:1915–1920. [PubMed: 15790844]

Bates JM, Mittge E, Kuhlman J, Baden KN, Cheesman SE, Guillemin K. Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. Dev. Biol. 2006; 297:374–386. [PubMed: 16781702]

Bates JM, Akerlund J, Mittge E, Guillemin K. Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. Cell Host Microbe. 2007; 2:371–382. [PubMed: 18078689]

Cao Y, Shen J, Ran ZH. Association between Faecalibacterium prausnitzii reduction and inflammatory bowel disease: A meta-analysis and systematic review of the literature. Gastroenterol. Res. Pract. 2014; 2014

Fisher CK, Mehta P. Identifying keystone species in the human gut microbiome from metagenomic timeseries using sparse linear regression. PLoS One. 2014; 9:e102451. [PubMed: 25054627]

Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc. Natl. Acad. Sci. USA. 2007; 104:13780–13785. [PubMed: 17699621]

Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Nat. Rev. Microbiol. 2012; 10:717–725. [PubMed: 22941505]

Harvie EA, Huttenlocher A. Neutrophils in host defense: new insights from zebrafish. J. Leukoc. Biol. 2015; 98:1–15. [PubMed: 26130765]

Hooper LV, Littman DR, Macpherson AJ. Interactions Between the Microbiota and the Immune System. Science. 2012; 336:1268–1273. [PubMed: 22674334]

Jemielita M, Taormina MJ, Burns AR, Hampton JS, Rolig AS, Guillemin K. Spatial and Temporal Features of the Growth of a Bacterial Species Colonizing the Zebrafish Gut. MBio. 2014; 5:1–8.

Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, Krakoff J. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am. J. Clin. Nutr. 2011; 94:58–65. [PubMed: 21543530]

Kanther M, Tomkovich S, Sun X, Grosser MR, Koo J, Flynn EJ, Jobin C, Rawls JF. Commensal microbiota stimulate systemic neutrophil migration through induction of Serum amyloid A. Cell. Microbiol. 2014; 16:1053–1067. [PubMed: 24373309]

MacIntyre DL, Miyata ST, Kitaoka M, Pukatzki S. The Vibrio cholerae type VI secretion system displays antimicrobial properties. Proc. Natl. Acad. Sci. USA. 2010; 107:19520–19524. [PubMed: 20974937]

Marino S, Baxter NT, Huffnagle GB, Petrosino JF, Schloss PD. Mathematical modeling of primary succession of murine intestinal microbiota. Proc. Natl. Acad. Sci. USA. 2013:1–6.

Mastropietro G, Tiscornia I, Perelmuter K, Astrada S, Bollati-fogolín M. HT-29 and Caco-2 Reporter Cell Lines for Functional Studies of Nuclear Factor Kappa B Activation. Mediat. Inflamm. 2015:1–13.

Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008; 453:620–625. [PubMed: 18509436]

Milligan-Myhre, K.; Charette, JR.; Phennicie, RT.; Stephens, WZ.; Rawls, JF.; Guillemin, K.; Kim, CH. Study of Host-Microbe Interactions in Zebrafish. Elsevier Inc.; 2011.

Newell PD, Douglas AE. Interspecies Interactions Determine the Impact of the Gut Microbiota on Nutrient Allocation in Drosophila melanogaster. Appl. Environ. Microbiol. 2014; 80:788–796. [PubMed: 24242251]

Oehlers SH, Flores MV, Okuda KS, Hall CJ, Crosier KE, Crosier PS. A chemical enterocolitis model in zebrafish larvae that is dependent on microbiota and responsive to pharmacological agents. Dev. Dyn. 2011; 240:288–298. [PubMed: 21181946]

Power ME, Tilman D, Estes JA, Menge BA, Bond WJ, Mills S, Daily G, Castilla JC, Lubchenco J, Paine RT. Challenges Quest for Keystones. Bioscience. 1996; 46:609–620.

Renshaw SA, Loynes CA, Trushell DMI, Elworthy S, Ingham PW, Whyte MKB. A transgenic zebrafish model of neutrophilic inflammation. Blood. 2006; 108:3976–3978. [PubMed: 16926288]

Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux J-J, Blugeon S, Bridonneau C, Furet J-P, Corthier G, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc. Natl. Acad. Sci. USA. 2008; 105:16731–16736. [PubMed: 18936492]

Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, Cosnes J, Corthier G, Marteau P, Doraé J. Low counts of faecalibacterium prausnitzii in colitis microbiota. Inflamm. Bowel Dis. 2009; 15:1183–1189. [PubMed: 19235886]

Sonnenburg JL, Chen CTL, Gordon JI. Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. PLoS Biol. 2006; 4:e413. [PubMed: 17132046]

Stein RR, Bucci V, Toussaint NC, Buffie CG, Rätsch G, Pamer EG, Sander C, Xavier JB. Ecological modeling from time-series inference: insight into dynamics and stability of intestinal microbiota. PLoS Comput. Biol. 2013; 9:e1003388. [PubMed: 24348232]

Stephens W, Burns A, Stagamann K, S W, Rawls J, Guillemin K, Bohannan B. The composition of the zebrafish intestinal microbial community varies across development. ISME J. 2015 10.1038/ismej.2015.140.

Tapia-Paniagua S, Vidal S, Lobo C, Prieto-Álamo M, Jurado J, Cordero H, Cerezuela R, García de la Banda I, Esteban M, Balebona M, et al. The treatment with the probiotic Shewanella putrefaciens Pdp11 of specimens of Solea senegalensis exposed to high stocking densities to enhance their resistance to disease. Fish Shellfish Immunol. 2014; 41:209–221. [PubMed: 25149590]

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, et al. A core gut microbiome in obese and lean twins. Nature. 2009; 457:480–484. [PubMed: 19043404]

Walters WA, Xu Z, Knight R. Meta-analyses of human gut microbes associated with obesity and IBD. FEBS Lett. 2014; 588:4223–4233. [PubMed: 25307765]

Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature. 2008; 455:1109–1113. [PubMed: 18806780]

Westerfield, M. The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish Danio (Brachydanio rerio). Eugene, OR: Institute of Neuroscience University of Oregon; 1993.

Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, Russell JM, Bevins CL, Adams LG, Tsolis RM, et al. Gut inflammation provides a respiratory electron acceptor for Salmonella. Nature. 2010; 467:426–429. [PubMed: 20864996]

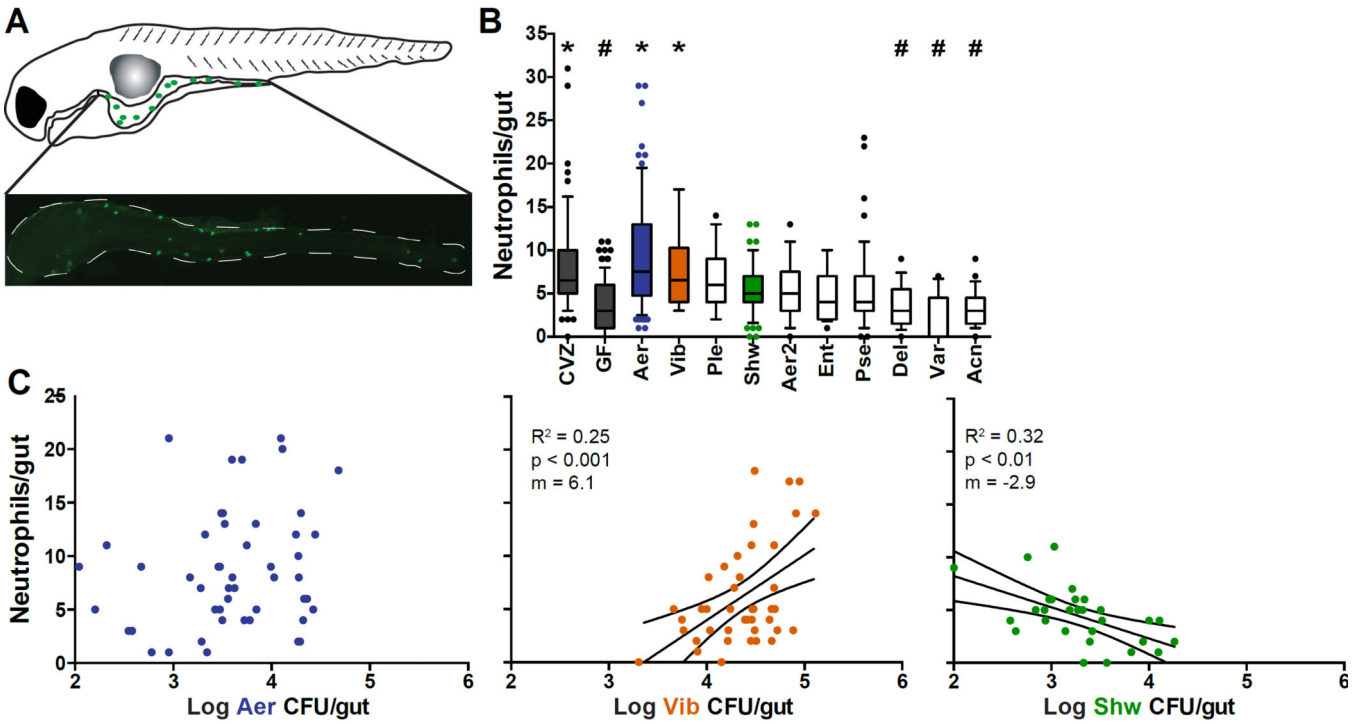

Fig. 1. Resident microbial isolates induce unique neutrophil responses

A. Dissected mpx:GFP zebrafish intestine. **B**. Intestinal neutrophil recruitment in conventionalized fish (CVZ), germ free fish (GF), or fish inoculated with ten individual mono-associations. Middle line, median; boxes, quartiles; whiskers, 10th–90th percentile. Aer, *Aeromonas;* Vib, *Vibrio;* Ple, *Plesiomonas;* Shw, *Shewanella;* Aer2, *Aeromonas sp. 2;* Ent, *Enterobacter;* Pse, *Pseudomonas;* Del, *Delftia;* Var, *Variovorax;* Acn, *Acinetobacter.* * Significantly different from GF; # significantly different from CVZ (ANOVA). **C**. Correlation between neutrophil influx and logarithm of bacterial abundance per intestine for *Aeromonas* (left), *Vibrio* (middle), and *Shewanella* (right). Linear regression analysis. For all conditions, N ≥ 20 fish, derived from at least three independent experiments.

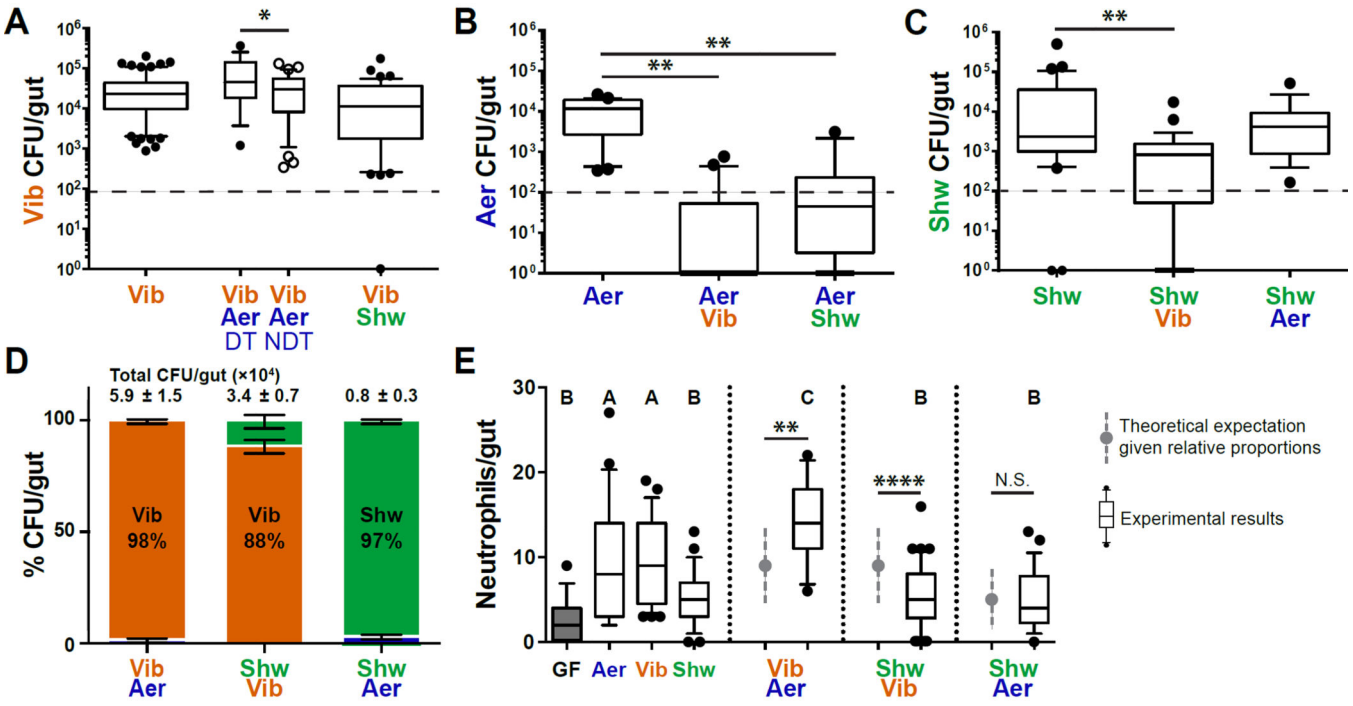

Fig. 2. Microbial di-associations reveal complex dynamics between microbes

A. *Vibrio* abundance in mono- or di-association with either *Aeromonas* or *Shewanella*. The *Aeromonas* di-association is split into two categories, *Aeromonas* detected (DT) or not detected (NDT) in the fish. **B.** *Aeromonas* abundance in mono- or di-association with either *Vibrio* or *Shewanella*. **C.** *Shewanella* abundance in mono- or di-association with *Vibrio* or *Aeromonas*. Dashed line indicates limit of quantification. For **A**, **B**, **C** *p < 0.05, **p < 0.01, ANOVA. **D.** Relative proportion of the total number of bacteria in di-associations (avg ± SEM). Total bacterial load is listed above bar graph. **E.** Intestinal neutrophil influx for each mono- or di- association. Conditions that share a letter are not statistically different, ANOVA. Grey dots and dashed lines represent the expectation for the di-associations given the relative proportion of species. **p < 0.01, **** p < 0.0001, one sample t test with theoretical mean; N.S., not significant. For all conditions, N ≥ 20 fish, derived from at least three independent experiments. See also Figure S1.

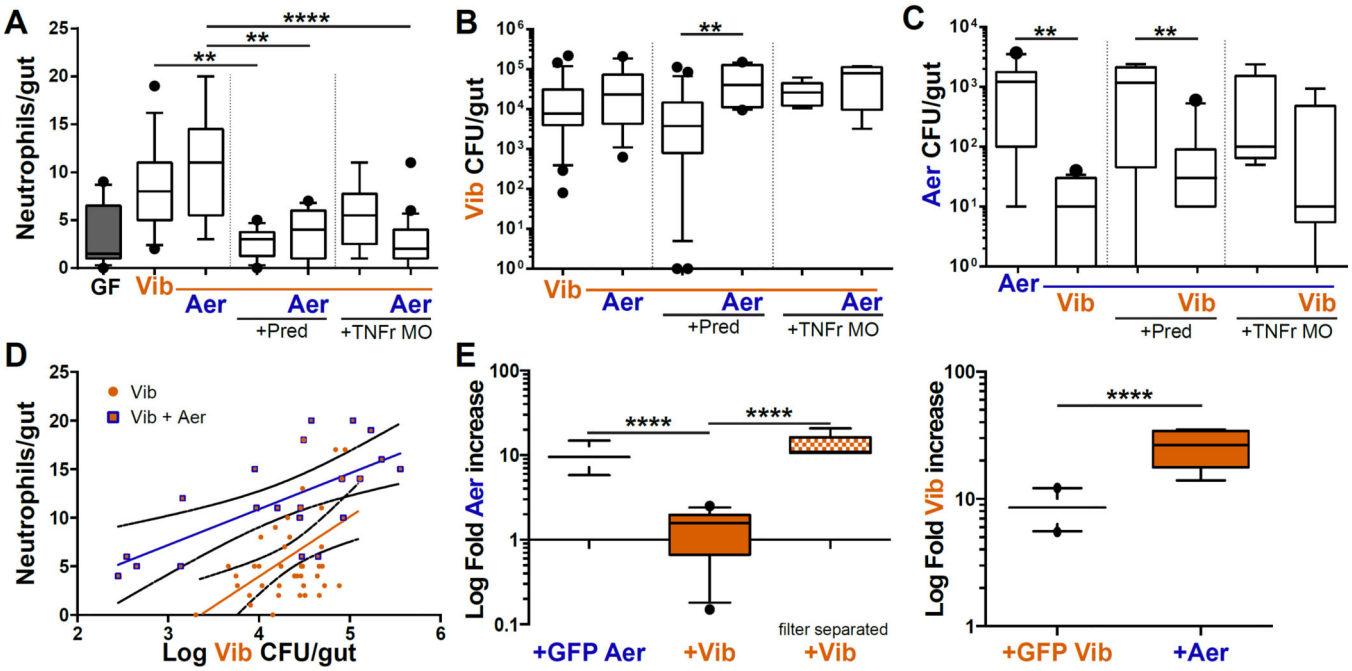

Fig. 3. An interaction between *Vibrio* and *Aeromonas* promotes *Vibrio* abundance

A. Immunosuppression via prednisolone (Pred) or the *tnfr* morpholino (TNFr MO) maintains neutrophil influx at GF levels in the presence of *Vibrio* mono- and di-associations. **B**. In the absence of a neutrophil response, *Vibrio* abundance increases in the presence of *Aeromonas*. **C.** *Aeromonas* abundance is reduced in the presence of *Vibrio* and is unaffected by prednisolone or *tnfr* morpholino treatment. **D.** The linear relationships between log(CFU) *Vibrio* abundance and neutrophil influx in mono- (orange circles) or in di-association with *Aeromonas* (blue and orange squares). **E.** Growth of *Aeromonas* and *Vibrio in vitro* either in co-culture with a GFP-tagged isogenic strain or mixed together on filter paper. *Vibrio* reduced *Aeromonas* growth (left), and *Aeromonas* increased *Vibrio* growth (right). Separating the species with filter paper prevented the reduction of *Aeromonas.* *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, T-test. See also Figure S2.

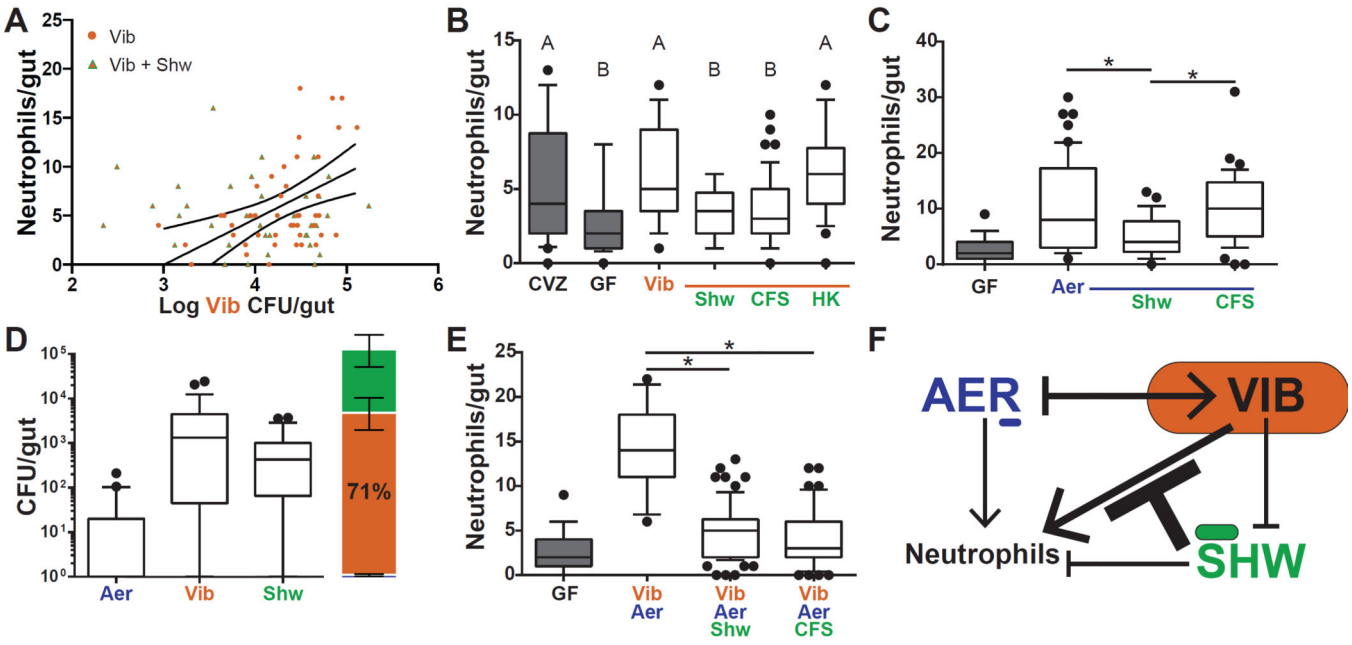

Fig. 4. *Shewanella* controls the neutrophil response via a secreted anti-inflammatory factor

A. The presence of *Shewanella* (green and orange triangles) abolishes the linear relationship between log(CFU) *Vibrio* (orange circles) abundance and neutrophil influx. Four independent experiments are displayed. **B.** Intestinal neutrophil influx in response to *Vibrio* with live *Shewanella*, *Shewanella* cell free supernatant (CFS), or heat-killed *Shewanella* (HK). Conditions that do not share a letter are significantly different (ANOVA, Tukey's range test). **C.** Intestinal neutrophil influx in response to a di-association of *Aeromonas* with either live *Shewanella* or *Shewanella* CFS. **D.** Abundance and percentages (avg ± SEM) of *Vibrio*, *Aeromonas*, and *Shewanella* in the tri-association. **E**. Neutrophil influx in response to the *Vibrio* and *Aeromonas* di-association, the tri-association, or the *Vibrio* and *Aeromonas* di-association with *Shewanella* CFS. *p < 0.05, T-test. **F**. Model of the inter-bacterial interactions occurring in the intestine that influence bacterial abundance and intestinal neutrophil influx. Arrow thickness denotes interaction strength; oval sizes reflect relative abundance in the tri-association. See also Figure S3.