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Breaking Down Walls: Microbiota and the Aging Gut

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A range of animal species show increased inflammation with age. In this issue of *Cell Host & Microbe*, Thevaranjan et al. (2017) reveal that heightened inflammation is associated with deregulation of homeostatic interactions between intestinal microbes and the aging host.

Chronic inflammation is a prognostic marker of increased disease risk and mortality in the elderly (Franceschi and Campisi, 2014). As such, characterizing factors that drive systemic inflammation is an important first step toward improving health with age. Elements causing age-related inflammation, however, remain mostly unknown. Since increased inflammation is also observed in aged insects (Clark et al., 2015; Li et al., 2016; Rera et al., 2012) and mice (Conley et al., 2016), researchers rely on these tractable model systems to uncover mechanisms of inflammation in aging animals.

Studies using *Drosophila* have provided valuable insight on the temporal mechanics of age-related inflammation, as classified by the activation of conserved signaling pathways that drive inflammation in mammals. Various studies have shown that the total number and composition of intestinal microbes shift in old flies—these changes are associated with misregulation of intestinal immune signaling and a breakdown in intestinal compartmentalization (Li et al., 2016) and barrier function (Clark et al., 2015). This disruption of the host-microbe association results in expansion of microbes into non-permissive areas, ultimately elevating systemic inflammation (Li et al., 2016), presumably as the host responds to translocated microbial products. As such, the state of host-microbe homeostasis is predictive of intestinal barrier function, which in turn is prognostic of fly mortality (Clark et al., 2015; Li et al., 2016; Rera et al., 2012).

These comprehensive studies using *Drosophila* were enabled by the genetic tools available for spatiotemporal control of gene expression and the organism's short lifespan. Consequently, factors crucial for intestinal, commensal, and organismal maintenance with age were identified in *Drosophila*, but until recently, studies connecting these factors in aged mammalian models were not conclusive.

The current study by Thevaranjan et al. (2017) in this issue of *Cell Host & Microbe* uncovers changes in mice that influence, or are associated with, age-related inflammation. Using aged mice, Thevaranjan et al. (2017) found that inflammation and immunocompetence are linked, and subsequently investigated the adverse effects and correlated factors of age-related inflammation. Macrophages sourced from the peritoneal cavity and bone marrow of aged mice were defective in combating *Streptococcus pneumoniae*. Since inflammation is associated with age (Franceschi and Campisi, 2014) and with hospitalization for pneumonia (Yende et al., 2013), Thevaranjan et al. (2017) hypothesized that the inflammation status of aged mice impacts anti-pneumococcal immunity. Indeed, older mice showed heightened pro-inflammatory cytokines when uninfected and following bacterial stimulation. Together, these data suggest that age-related inflammation reduces the anti-bacterial activity of macrophages.

What influences systemic inflammation in aged mice? The subsequent experiments of Thevaranjan et al. (2017) were based on a long-standing hypothesis recently supported in *Drosophila* that intestinal microbes expand into non-permissive areas as intestinal barrier function declines with age (Franceschi and Campisi, 2014). Hosts then mount inflammatory responses against bacterial moieties encountered in circulation. To determine if enteric microbes factor into their study, Thevaranjan et al. (2017) tested gut barrier function and found evidence of increased permeability in a portion of the gut containing the highest number of microbes, suggesting that greater intestinal permeability could allow bacteria or bacterial products into circulation. Consequently, the authors found greater levels of muramyl dipeptide, a bacterial cell-wall component and perhaps a signature of displaced microbes, circulating in plasma of aged mice. Permeability changes were also reported in the same mouse strain colonized with a different, limited set of bacteria, suggesting that barrier dysfunction is a general phenomenon that is associated with bacterial translocation from the intestine into the systemic bloodstream with age.

The current study concludes by separately testing how enteric microbes and inflammation function in the identified aging phenotypes. Aged mice born and raised germ free showed no decline in age-associated measures, revealing that the absence of microbial factors is associated with maintenance of intestinal integrity and low systemic inflammation. Subsequent experiments suggest that the aged microbiota causally influences these phenotypes, since young germ-free mice colonized with enteric microbes from old donors showed increased intestinal permeability and systemic inflammation. The negative impacts on host enteric and systemic physiology indicate that age-related microbe composition changes are dysbiotic. Although the current report qualitatively measured alterations in microbial composition, specific dysbiotic changes underlying barrier dysfunction were not identified.

Thevaranjan et al. (2017) also investigated the role of inflammation in their

age-associated measures using mice deficient in the pro-inflammatory cytokine, tumor necrosis factor (TNF). Aged TNF knockout mice did not show increased systemic inflammation with age and maintained intestinal barrier function. Importantly, age-associated microbial changes were less evident in TNF knockout animals, and administering anti-TNF therapy to aged conventional mice modulated microbial diversity, suggesting that TNF-mediated inflammation underlies alterations in intestinal permeability and microbial composition with age. It is not yet known, however, if overexpression of TNF is sufficient to drive these age-associated phenotypes.

Overall, Thevaranjan et al. (2017) demonstrate that age-associated microbial dysbiosis in mice promotes intestinal barrier dysfunction and systemic inflammation; lowered inflammatory capability prevents or reduces at least some of these malfunctions. The comparative use of germ-free mice was a highlight of the current report, but the maintenance of germ-free mouse colonies necessitated separate facilities and diets and thus introduced slight variation in conditions between conventional and germ-free animals. Nonetheless, the studies reveal the extent to which microbiota-associated changes impact health in aged animals—a greater number of germ-free mice lived to 600 days compared to conventional animals. While the current study adds to a growing body of literature demonstrating that late-life dysbiotic changes in intestinal microbiota are a negative occurrence in a range of animals (Clark et al., 2015; Conley et al., 2016; Li et al., 2016), it is important to note that enteric microbes are fundamental for proper metabolism and immune system development (Hooper et al., 2012). As such, future studies might focus on causative factors of age-associated dysbiosis and discovering ways to maintain homeostatic intestinal microbes with age.

An immediate approach could be to identify causative microbial shifts. Through a targeted sequencing approach, the current study measured changes to microbial composition at the genus level, but specific changes detrimental to the aged host were not clear. Future studies may benefit from broader whole-genome shotgun sequencing for more detailed taxonomic classification of enteric microbial shifts (Ranjan et al., 2016). It is not known if specific microbial species drive age-related malfunctions—evidence suggests that many bacterial strains can generate comparable effects on adiposity and the host immune system (Faith et al., 2014). It could be that multiple strains of bacteria underlie the reported pathologies with age. Alternatively, changes to the total number or the composition of microbes, or both, may be critical determinants of host health.

Although germ-free animals were protected from age-related inflammation and intestinal barrier dysfunction, age still sensitized these animals to microbial exposure. Old axenic mice showed heightened TNF upon exposure to microbes, regardless of whether the donors were young or old. Further studies will be needed to determine if these age-related changes to microbial sensitivity extend beyond cytokine responses.

The pathologies outlined in the current report parallel age-associated phenotypes in the fly, suggesting that high-throughput fly studies can be used to complement mammalian studies on transformations of aging host-microbe systems. Future research may benefit from a multifaceted approach focused on both microbe- and host-specific alterations in complementary fly and mouse models. Such studies may allow for a deeper characterization of the complex physiological changes impacting microbial homeostasis, the sensitivity to microbial constituents in aged hosts, and organismal decline with age.

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