AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

MEETING REVIEW

The Costs of Living Together: Immune Responses to the Microbiota and Chronic Gut Inflammation

Lucas J. Kirschman,[a] Kathryn C. Milligan-Myhre[a]

[a]Department of Biological Sciences, University of Alaska—Anchorage, Anchorage, Alaska, USA

ABSTRACT While the vertebrate microbiota is critical to the normal function of many host traits, hosts may expend a large amount of energy to constrain and interface with their microbiota via their immune system to avoid the high fitness costs associated with gut dysbiosis, pathobionts, and opportunistic pathogens. All jawed vertebrates share mucosal immunity dedicated to isolating the microbiota, and a breakdown of this system can result in chronic gut inflammation. In humans, chronic gut inflammation negatively affects growth and development. There is little information available on the prevalence of chronic gut inflammation in wild animals, but given that animals with different life histories emphasize different immune responses, it follows that wild animals may vary in their susceptibility to chronic gut inflammation, and most animals will experience signaling that can lead to this state. These can be top-down signals originating from sources like the central nervous system or bottom-up signals originating from changes in the gut microbiota. The sources of these signals might include stress, developmental transitions, food restriction, and dietary shifts. Here, we briefly discuss host-microbiota interactions from the perspective of life history theory and ecoimmunology, focusing on the mucosal immune system and chronic gut inflammation. We also include future directions for research and the tools necessary to investigate them.

KEYWORDS ecological immunology, physiological trade-off, glucocorticoids, mucosal immunity, neuroendocrine stress response

The microbiota inhabiting the vertebrate gut is crucial to much of host physiology (1–4). The immune system must interface with the microbiota and defend against pathogens. The specifics of these interactions have been the subject of many reviews (5–7). Immune responses can carry a large energetic cost and often come at the expense of other life history traits, because no animal can maximize all their traits (8–12). Vertebrate hosts may expend substantial resources to constrain and interface with gut microbiota, considering the high fitness costs associated with gut dysbiosis, pathobionts, and opportunistic pathogens (2, 13, 14). Indeed, subinhibitory doses of antibiotics promote growth in several domestic vertebrate taxa and in two wild-bird populations by suppressing growth and virulence factors of the gut microbiota, likely reducing the strength and cost of the immune response necessary to constrain it (15–19). In this review, we provide a brief discussion of what is known about host-microbiota interactions from the perspective of life history theory and ecoimmunology, the investigation of variation of immune responses (20). We focus on the mucosal immune system and the costs and causes of chronic inflammatory responses in the gut. We conclude with questions to address and the tools necessary to investigate them.

HOST IMMUNE RESPONSES

Complexity of host immune response and life history evolution. The vertebrate immune system is a complex collection of interacting immune factors, rather than a

Citation Kirschman LJ, Milligan-Myhre KC. 2019. The costs of living together: immune responses to the microbiota and chronic gut inflammation. Appl Environ Microbiol 85:e02147-18. https://doi.org/10.1128/AEM.02147-18.

Editor Eric V. Stabb, University of Georgia

Address correspondence to Lucas J. Kirschman, ljkirschman@alaska.edu.

Accepted manuscript posted online 7 December 2018
Published 2 May 2019

single trait defined as "immune function" (21, 22). Vertebrate innate immune factors are mostly nonspecific defenses (21–23), including anatomical boundaries, humoral factors, and cellular responses (21, 24). Innate factors are relatively inexpensive to maintain, but their activation, particularly cellular responses, carries large energetic and nutritional costs (8, 21, 25). Furthermore, cellular and humoral innate factors are often nonspecific and risk self-damage (21, 26). This potential for self-damage is particularly true of the inflammatory response, in which increased permeability of vascular-endothelial boundaries allows leukocytes and proteins to travel into affected tissues (26). The damage caused by inflammation underlies the pathophysiology of many diseases (27). In contrast, adaptive immune factors, mediated by lymphocytes, are more specific and slower, although they allow for faster responses upon subsequent exposure to the same pathogen and are less likely to cause self-damage (21, 26, 27). However, adaptive immune responses are costly to develop, maintain, and use because of the expense of lymphocyte proliferation and somatic hypermutation (21, 28, 29).

Vertebrates emphasize different immune factors, based on demands specific to their life history (21). An animal's life history encompasses birth, growth, reproduction, and death (30, 31). Natural selection acts on traits, discrete aspects of an animal's phenotype, such as immune function. However, increasing the performance of one trait comes at the expense of another, due to limited resource pools; this limits trait combinations (32, 33). For example, the strength of any one immune factor does not necessarily correlate with fitness, because the cost of activating an immune factor may exceed its benefit (8, 9, 25, 27, 34, 35).

The pace-of-life hypothesis predicts that a species' life history should affect traits like immunity. Species exhibiting a "slow" pace of life, with long developmental times and life spans, are more likely to invest in relatively expensive adaptive immune responses, because they are pathogen specific and less likely to inflict self-damage (21, 36). "Fast-paced" species, with short developmental times and life spans, may exhibit weaker adaptive immune responses because of the high costs of lymphocyte proliferation and activation (21, 28). Fast-paced species likely rely on the innate immune response because the maintenance costs are low, but if an initial response does not clear a pathogen, there is a risk of an expensive, self-damaging, inflammatory response (8, 21). Phylogenic differences in metabolism can also affect immunity. Ectotherms with low mass-specific metabolisms may lack the energy for rapid somatic hypermutation and antibody responses (29). For example, reptile antibody titers and binding affinity do not increase with secondary pathogen exposure, and the binding affinity of mammalian antibodies increases orders of magnitude more than those of amphibians exposed to the same challenge (37, 38). These weaker adaptive responses may occur because the lymphoid tissue of amphibians and reptiles is relatively simple, lacking lymph nodes and germinal centers (37, 38), but a definitive mechanism for these differences remains unclear.

Explanations of life history trade-offs between vertebrate immune factors have largely focused on their ability to defend against microbial threats (21, 22). However, the microbiota contains many beneficial microbes, pathogens, and pathobionts (39). A healthy microbiota plays immunological roles both by stimulating host immunity and via interactions with pathogens. For example, colonization of commensal strains of *Escherichia coli* in the gut stimulates the development of intestinal T cells and inhibits the growth of pathogenic strains of *E. coli* (40). An optimal immune response to the microbiota should sequester harmful microbes and their by-products, allow passage of useful substances, and avoid damage of self or beneficial microbes. This response is also likely continuously active, because the gut microbiota is always present (5–7). There is no reason to suspect that an organism can attain this optimal immune response, and thus, sequestration, allowing passage, and avoiding damage are likely competing traits. Understanding how the immune response to the microbiota governs the balance between these traits may be crucial to life history evolution, because these dynamics may underlie other crucial traits such as development, growth, and metabolism (16, 17, 41–44).

Vertebrate mucosal immunity and conservation of antibodies. The gut mucosal immune system must isolate microbes, particularly pathogens and pathobionts, while limiting the activation of the systemic immune system in response to commensal and symbiotic organisms (45). These selective pressures, particularly the high cost of inflammation, have driven the evolution of dedicated gut mucosal immunity in vertebrates (46–48).

The intestinal epithelium sequesters the microbiota and prevents microbes from escaping the gastrointestinal (GI) tract. Intestinal epithelial cells are polarized to reduce proinflammatory signaling from the lumen while promoting inflammation if signals originate from basolateral surfaces (45). They also form tight junctions that restrict transport across cell layers and secrete mucus and antimicrobial peptides, all of which control microbial organization (49–51). Adaptive immune responses aid in the sequestration of the microbiota.

All major clades of jawed vertebrates, save cartilaginous fish, have evolved immunoglobulins (Igs) specific to the mucosal immune system that help prevent microbes from crossing the intestinal epithelium (5, 7, 38, 46–48, 52–54). IgA is present in mammals, birds, and crocodilians; is well described due to its role in human GI health; and descended from amphibian IgX (5, 6, 55). Some species of snakes, lizards, and turtles have lost IgX and IgA orthologues, and it is unclear why losses occurred in these clades (38, 47, 48, 52). However, some species that lack IgA may use IgM as a substitute (53). Bony fish have evolved IgT independent of the tetrapod Ig lineage (46, 54). A complete review of the coevolution of mucosal Igs is beyond the scope of this review. However, Kaetzel (48) provides an exhaustive review of mucosal Igs and suggests that selective pressures favored the evolution of transmembrane proteins that transport Igs across intestinal epithelia without compromising epithelial barrier integrity and that the complexity of IgA evolved to protect the molecular structure against proteolytic attack.

Despite similarities in the mucosal epithelium and Igs, other aspects of mucosal immunity differ across taxa (54, 55). For example, the gut-associated lymphoid system (GALT) in mammals is concentrated into lymphoid follicles, such as Peyer's patches. Fish, amphibians, and reptiles lack these structures; the lymphoid follicles that make up their GALT are more spread out, possibly because ectothermic metabolisms cannot support rapid somatic hypermutation and lymphocyte proliferation (29, 38, 45, 54, 55). Mammals also have more diverse classes of innate lymphoid cells and more dendritic cells present in their mucosa (54, 55).

Gut mucosal immunity in vertebrates seems to carry a substantial energetic cost. For example, subinhibitory doses of antibiotics promote growth in livestock and two species of wild birds by negatively affecting the microbiota and allowing animals to invest in somatic growth rather than mucosal immunity (15–19). Chickens also grow larger under germfree conditions, presumably because they are less reliant on their microbiota to extract energy and nutrition from food than mammals and fish (16). However, the cost of gut mucosal immunity is lower than that of the chronic gut inflammation that can occur if microbes or their associated peptides pass the intestinal epithelium (49–51, 56).

Chronic gut inflammation and pace of life. Chronic gut inflammation, which presents in humans as inflammatory bowel disease, is one of the most studied diseases related to the human microbiota, and this state can also be induced in vertebrate models (57, 58). Chronic gut inflammation occurs when the innate and adaptive arms of the systemic immune system respond to microbes, or their associated peptides, that have crossed the intestinal epithelium (59–61). Information on the frequency of chronic gut inflammation in wild animals is limited. There is evidence from wild mice that stabilizing selection acts on host immune responses that constrain the microbiota, because hybrid house mice (*Mus musculus*) exhibit altered microbiota and pathology consistent with chronic gut inflammation (62). Information on the prevalence of such pathology in wild animals may be important data for ecoimmunological theory, because chronic gut inflammation is associated with life history trade-offs, specifically

reduced growth and delayed puberty, independent of changes in nutrient absorption (63, 64). If chronic gut inflammation is common in wild populations, it could represent an understudied energetic and nutritional cost and a potential source of life history variation. However, the immunological and energetic consequences of chronic gut inflammation may vary across vertebrates, because differences in host life histories and immune factors could affect systemic immune responses to the microbiota.

Fast-paced vertebrates and ectotherms that rely on innate immunity may be more likely to initiate a systemic inflammatory response to the microbiota if a reliance on innate immunity means that they have more innate immune cells in their intestines. Innate cells in the intestinal mucosa can begin a signaling cascade that recruits the adaptive immune system and inflammatory processes (60). However, without strong adaptive immunity, the inflammation may be weak or short-lived. Adaptive immune factors, specifically the balance of Th1 and Th2 cytokines, mediate the strength and duration of inflammatory responses in the gut (60). Thus, slow-paced species, and perhaps endotherms whose metabolisms can support stronger adaptive immune responses (29), may be less likely to initiate an inflammatory response to their microbiota but suffer longer and more-intense gut inflammation when it does occur. Mammals, for example, exhibit critical windows in early development where shifts in the gut microbiota are more likely to result in autoimmune diseases, including chronic gut inflammation (50, 65, 66).

It is difficult to predict how pace of life may affect gut inflammation, because the data on gut inflammation and mucosal immunity in nonhuman animals are mostly limited to mammalian laboratory models (67), which may limit the applicability of these results to other taxa. There have been some efforts to expand these investigations to other vertebrate hosts (54, 55, 57, 68, 69), but these are mostly laboratory based. We suggest that the next step is to explore the natural causes of gut inflammation in populations.

It is unclear whether chronic gut inflammation is influenced primarily by top-down interactions, where changes in endocrine signaling induce alterations in gut morphology and immune responses, or by bottom-up interactions, wherein changes in the gut microbiota induce maladaptive immune responses (70). Regardless, animals regularly experience circumstances that can alter top-down signaling, like environmental stressors and developmental transitions, and bottom-up signaling, such as dietary changes, starvation, and fasting (71–77). In the following section, we review some potential causes of chronic gut inflammation as a guide for future inquiry.

TOP-DOWN SIGNALING: THE HPA/I AXIS AND CHRONIC GUT INFLAMMATION

Vertebrates mediate many of their physiological responses to environmental stressors via the conserved hypothalamic-pituitary-adrenal/interrenal (HPA/I) axis (78). The hormones secreted by this axis can regulate many traits that are often involved in life history trade-offs, such as immune function, development, growth, metabolism, and nutrient balance (79–83). The HPA/I axis is also central to understanding gut inflammation and therefore may mediate some microbiota effects on animal life history.

Hormonal stimulation of gut inflammation. Part of the larger brain-gut (BG) axis, the HPA/I axis has effects on gut physiology and host-microbiota communication (84). The HPA/I axis affects the inflammatory state of the gut via actions of corticotropin-releasing factor (CRF) and glucocorticoid (GC) hormones, altering the BG axis at several levels of organization (49, 56, 85, 86). CRF promotes inflammation by increasing intestinal permeability, activating immune cells, and inhibiting anti-inflammatory pathways of the vagus nerve (49, 56, 85, 86). CRF also slows gastric emptying and small intestinal motility while promoting motility in the colon (86). CRF's downstream actions lead to the release of GC hormones, which suppress appetite (80, 87). Although GC hormones typically have anti-inflammatory actions, these effects do not seem to counteract the inflammatory action of CRF on the BG axis (49, 82). There is some evidence that GC hormones increase intestinal epithelial permeability and bacterial adherence to intestinal mucosa (88).

The effects of HPA/I axis activation can alter host physiology and the microbiota and lead to chronic gut inflammation. However, changes in the microbiota can also increase intestinal epithelial permeability and elicit an inflammatory response (70). The pleiotropic effects of the HPA/I axis on other aspects of host physiology and life history could increase the chances or exacerbate the consequences of a maladaptive inflammatory response in the gut, particularly in young and developing animals.

The HPA/I axis, development, and the microbiota. Young and developing animals are often more susceptible to pathogens and pathobionts, due to their naive immune systems (37, 89, 90). Additionally, the HPA/I axis mediates trade-offs between development and somatic maintenance that can further weaken immune responses (83, 91–94). During this time period, many animals also undergo dietary and ontogenetic changes that exert strong effects on microbiota community structure (71, 95–97).

Amphibian larvae present an informative case study of these effects. In late-stage amphibian larvae, GC hormones synergize with thyroid hormones to accelerate development (87). This allows larvae to escape poor-quality aquatic habitats by metamorphosing (87, 98, 99). However, accelerated development comes at a cost. Shorter larval periods constrain the time available for growth, and HPA/I activation allocates resources toward development at the expense of growth and immune function (91, 92, 100). The microbiota can moderate this trade-off by changing the size of the energy pool. For example, wood frog (*Lithobates sphenocephalus*) larvae inoculated with bullfrog (*Lithobates catesbeianus*) microbiota both developed and grew faster than wood frog larvae inoculated with microbiota from conspecifics (182). These results imply that the microbiota of wood frog larvae inoculated from bullfrogs provided more energy, perhaps owing to the increased presence of anaerobic fermentative bacteria (182).

It is possible that amphibians and other vertebrates with complex life histories, where morphology, physiology, habitat, and diet differ greatly between life stages, are more tolerant of microbiota alterations during early development and metamorphosis because their life histories include radical shifts in physiology, diet, habitat, and, thus, their associated microbes. In addition, metamorphosis constrains many physiological processes, including immune function. Larvae downregulate multiple immune factors during metamorphic climax to avoid attacking newly formed adult tissue (37, 101–104). As a result, the immune system of larval amphibians may be unlikely to respond to changes in the gut microbiota by initiating a chronic inflammatory response. It seems possible that animals with more-predictable microbial environments could have more-maladaptive responses to microbiota alterations.

Microbiota transplants between vertebrate species with simpler life histories have affected metabolism and obesity phenotype. Microbiota from human fecal material successfully colonized mice and altered metabolic pathways, gene expression, and adiposity (105). Altering the microbiota of preweaned mice with subtherapeutic levels of antibiotics produces similar phenotypes that persist after the reestablishment of a normal microbiota (106). While faster development and larger size at metamorphosis are adaptive in amphibians (99, 107–110), this is not true of increased adiposity in mice. Indeed, this phenotype in mice is associated with increased gut permeability, weaker mucosal responses in the gut, and changes in systemic inflammatory responses (106, 111).

BOTTOM-UP SIGNALING: DIET AND CHRONIC GUT INFLAMMATION

The immune system can affect microbiota community structure by acting as a filter during community assembly (112, 113). However, these effects may be weak in comparison to other aspects of community assembly, such as the environmental species pool (113, 114). Diet seems to be a strong driver of microbiota community structure on individual, population, and species levels (72, 77, 115–120). The diet of animals can vary considerably between habitats and seasons, and the vertebrate gut microbiota can strongly influence host metabolism, nutrition, and the inflammatory state of the gut (121–126). Changes in diet are associated with chronic gut inflamma-

tion in mice, humans, and fish (54, 127, 128). For animals, changes in food type and availability are common (129–131). Both of these could affect the microbiota and the host's immune response (34, 126).

Food restriction: starvation and fasting. Animals often experience bouts of food limitation. Even single instances of food deprivation can initiate responses that can affect the gastrointestinal tract and the microbiota (132, 133). However, there is a distinction between starvation, caused by extrinsic forces, and fasting, initiated by internal stimuli (132). For example, a period of food deprivation during winter is starvation, while hibernation through winter is fasting. The adaptations and acclimations that help animals survive starvation differ from those that allow them to fast (132).

Starvation's effects on physiology are dependent on the length of food deprivation (134). While chronic starvation can lead to protein catabolism and death (134), low food availability, short-term food deprivation, or intermittent starvation instigates changes that allow animals to extract maximum energy from food. The gastrointestinal tract can maintain its mass, or even enlarge, despite decreases in body mass (135). Additionally, the absorptive area of the small intestine can increase, and the mucin layer thins, allowing for increased glucose and lipid transport across the intestinal epithelium (135–138). For the microbiota, these are drastic habitat alterations, coupled with a lack of energetic and nutritional inputs. Starvation causes significant changes in the microbiota community, but this varies by host taxa, likely reflecting differences in the initial microbiota community or host physiology (133).

Caloric restriction also activates the HPA/I axis (139). All of this indicates that starvation could cause chronic gut inflammation. This could again affect hosts differently depending on their life history, because the strength of vertebrate immune factors is limited by many micro- and macronutrients that are limited during starvation (8, 139, 140). For example, zinc, vitamin A, and caloric intake can affect the balance of T-lymphocyte responses (28), and cellular responses, from both the innate and adaptive arms, require amino acids (8).

Research into the effects of starvation should examine the microbiota and the strength of mucosal immune factors during starvation as well as how they recover with the restoration of food. There is evidence that microbes are critical to reestablishing gut health. For example, live *Lactobacillus murinus* bacteria increased colonic epithelial cell proliferation of starved mice following refeeding (141). It would also be valuable to investigate these dynamics in species that are adapted for periodic starvation versus those that are not, because their physiological responses may be different (132). For example, the gastrointestinal tracts of animals that feed intermittently can undergo considerable morphological and physiological changes following a meal (142).

The physiological and microbial responses to fasting are distinct from those for starvation (132, 143). Birds can endure fasting during migration by fueling their flight mainly on lipid reserves. (144). Hibernating bears reduce their energy expenditure by half, preserve muscle density, and recycle almost all of the nitrogen in their urea. Ureolytic microbes present in the bear microbiota are crucial to this process and may also contribute to a "healthy obesity phenotype" (145, 146). Microbial ureolysis in hibernating amphibians has also been documented (147). Ground squirrels exhibit distinct winter microbiota with similar microbial communities occurring in different species, dominated by microbes that survive on endogenous host resources (148, 149). This may represent a specialized, hibernatory microbiota (148). It would be valuable to track the mucosal immune responses of animals that undergo periodic fasts to see if and how they suppress gut inflammation and how these responses differ from those for starvation.

Diet shifts. The microbiota is integrated in metabolism and nutrient uptake and can allow for ecological acclimation and adaptation to nutritional landscapes (121, 150, 151). Subpopulations of fire salamander (*Salamandra salamandra*) larvae exhibited no changes in growth rate when transplanted between stream and pond habitats, possibly because rapid changes in their microbiota allowed them to extract nutrition from

different food sources (74). Similarly, microbiota shifts in animals that experience seasonally varied food resources allow them to compensate for fluctuations in energy and nutrient availability (152–155). Despite adaption to pond or streams, salamander larvae are generalist, gape-limited predators (74). The mucosal immunity of generalists and animals with seasonally varied diets may be less likely to initiate an inflammatory response when confronted with shifts in the microbiota.

In contrast, the microbiota of specialist herbivores such as woodrats (genus *Neotoma*) and the greater sage-grouse (*Centrocercus urophasianus*) allow their hosts to exploit otherwise toxic food sources (156, 157). These host-microbe relationships seem closer than those of generalists. Woodrat microbiota directly detoxify food and stimulate detoxifying enzymes in the liver (157). They also retain about 60% of their wild microbiota in captivity, and microbiota transplants can confer detoxification ability on naive congeners (157). Since the immune system can act as a filter for the microbiota (112, 113), it would be interesting to see if the immune systems of woodrats and other dietary specialists select for the colonization of mutualistic, detoxifying microbes.

TOOLS AND AREAS OF CONSIDERATION FOR ECOIMMUNOLOGICAL STUDIES

Quantifying the cost of mucosal immunity and chronic gut inflammation and tying these costs explicitly to physiological trade-offs and fitness require some methods familiar to ecoimmunologists (23) and some that may not be. Assays that assess the strength of systemic immune responses (23, 158, 159) may not correlate with the state of mucosal immunity and the microbiota. Ideal methods for measuring chronic gut inflammation should quantify the inflammatory responses within the gastrointestinal tract. A direct method is counts of innate immune cells within the intestines. Intestinal cross sections can be slide mounted and stained, and immune cells can be counted manually (68). Slide-mounted cross sections also allow measurement of histological damage associated with chronic gut inflammation (160). Manual counts are cost-effective but labor-intensive. Flow cytometry costs more but provides higher throughput and allows for simultaneous measurements of phagocytosis and respiratory burst (160, 161). An advantage of both types of cell counts is that they are applicable to many species. Gene expression assays, such as quantitative PCR, the microarray technique, and RNA sequencing, can also provide a quantitative measure of inflammation with simple comparison to a baseline state or control group (162). However, these assays are expensive and require tightly controlled, standardized workflows for accurate results (163–165). The disadvantages of cell counts and gene expression are that they are laboratory based and sacrificial. They preclude longitudinal investigations, may complicate field studies, and are impractical for large or endangered species.

Nonlethal markers may provide indirect measures of gut inflammation (166), but these markers were developed as human diagnostic tools. Extending use to animal models requires validation. For example, C-reactive protein levels in the blood rise during inflammatory responses, and chronically elevated levels can be indicative of chronic gut inflammation in humans (166). However, blood levels that indicate disease in humans likely do not translate to animal models, especially for wild populations that may exhibit variance in inflammation in response to parasitism. Ecoimmunologists seeking indirect methods to detect chronic gut inflammation should consider fecal calprotectin levels. Calprotectin represents the majority of granulocyte cytosolic proteins and correlates directly with intestinal permeability and neutrophils in the gastrointestinal tract (166–168). Calprotectin can also remain stable in feces for about a week at room temperature (166). While used widely in human studies, this could be adapted for use in wild-animal models relatively simply, as has been done in laboratory-raised rats (169).

Beyond measurements of gut inflammation, a practical first step for investigating the cost of mucosal immunity is administration of subinhibitory doses of antibiotics to wild populations (18, 19). Such studies could be performed across multiple species and life stages, providing information on what traits are negatively associated with mucosal immunity and providing a direct measurement of fitness costs to reproductive adults.

Another potential area of inquiry is comparisons of gut inflammation with the endogenous level of systemic immune responses, as these arms of the immune system may compete for resources.

Researchers should keep in mind that mucosal immune responses have a healthy basal state where the host is successfully constraining the microbiota (170). Even high levels of gut inflammation may be adaptive if they occur in response to enteric pathogens (63, 170). Additionally, genes associated with gut inflammation may confer advantages to animals during reproductive life stages and exact costs later, when morbidity and mortality have less of an impact on fitness (63, 170, 171). Therefore, tools that compare both induction and basal states of the immune responses in the gut would be beneficial.

Finally, studies of mucosal immunity and gut inflammation should include measurements of the microbiota. There are multiple methods to sequence and measure the microbiota (172–174); a full discussion is beyond our scope. However, investigations that account for microbiota community structure should take the Anna Karenina hypothesis into account: healthy microbiotas are similar, but transitions to an unhealthy microbiota are stochastic and may be difficult to detect (2). Additionally, just as we have increased our knowledge of the microbiota of model organisms, from *Drosophila* to humans (175–178), we must also identify, characterize, and create databases for the microbiota of nonmodel organisms. This task is increasingly feasible with next-generation sequencing (179). However, there is a need to compare the microbiota, host genetic background, and immune markers simultaneously and nonlethally, especially for rare species and wild populations, and there are few nonlethal methods for extracting high-quality RNA and DNA from a single sample. This may limit longitudinal studies across the dietary, physiological, and ecological shifts described in this review. Thus, researchers should carefully consider their questions and the methods available to answer them.

CONCLUSIONS

In recent decades, ecoimmunology has emerged as an integrative field and may help inform forces driving life history evolution (158). At the same time, our understanding of the microbiota and its important role in human health, immune function, and evolutionary theory has increased (3, 39, 180, 181). The microbiota is an integral part of metabolism and the vertebrate immune system, a source of pathogens and pathobionts, and a potential trigger for chronic inflammatory responses. Ecoimmunological investigations into the cost of immune responses and their negative effects on other traits cannot afford to ignore the interaction between host mucosal immune responses and the microbiota. Indeed, combining the methods and hypotheses of these two fields has the potential to include these proximate interactions between the microbiota and the immune system, particularly the costs of chronic gut inflammation, into the frameworks of developmental trade-offs and dietary evolution.

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