Microbiome and pathogen interaction with the immune system

Michael H. Kogut,[*,1] Annah Lee,[*,†] and Elizabeth Santin[‡]

*Southern Plains Agricultural Research Center, USDA-ARS, College Station, TX, 77845 USA; †Department of Poultry Science, Texas A&M University, College Station, TX, 77845 USA; and ‡Universidade Federal Do Paraná, Department of Veterinary Medicine, Curitiba, 80035-050 Brazil

ABSTRACT The intestinal tract harbors a diverse community of microbes that have co-evolved with the host immune system. Although many of these microbes execute functions that are critical for host physiology, the host immune system must control the microbial community so that the dynamics of this interdependent relationship is maintained. To facilitate host homeostasis, the immune system ensures that the microbial load is tolerated, but anatomically contained, while remaining reactive to microbial invasion. Although the microbiota is required for intestinal immune development, immune responses regulate the structure and composition of the intestinal microbiota by evolving unique immune adaptations that manage this high-bacterial load. The immune mechanisms work together to ensure that commensal bacteria rarely breach the intestinal barrier and that any that do invade should be killed rapidly to prevent penetration to systemic sites. The communication between microbiota and the immune system is mediated by the interaction of bacterial components with pattern recognition receptors expressed by intestinal epithelium and various antigen-presenting cells resulting in activation of both innate and adaptive immune responses. Interaction between the microbial community and host plays a crucial role in the mucosal homeostasis and health status of the host. In addition to providing a home to numerous microbial inhabitants, the intestinal tract is an active immunological organ, with more resident immune cells than anywhere else in the body, organized in lymphoid structures called Peyer's patches and isolated lymphoid follicles such as the cecal tonsils. Macrophages, dendritic cells, various subsets of T cells, B cells and the secretory immunoglobulin A (IgA) they produce, all contribute to the generation of a proper immune response to invading pathogens while keeping the resident microbial community in check without generating an overt inflammatory response to it. IgA-producing plasma cells, intraepithelial lymphocytes, and γδT cell receptor-expressing T cells are lymphocytes that are uniquely present in the mucosa. In addition, of the γδT cells in the intestinal lamina propria, there are significant numbers of IL-17-producing T cells and regulatory T cells. The accumulation and function of these mucosal leukocytes are regulated by the presence of intestinal microbiota, which regulate these immune cells and enhance the mucosal barrier function allowing the host to mount robust immune responses against invading pathogens, and simultaneously maintains immune homeostasis.

Key words: microbiota, innate immunity, gut health, *Salmonella*, mucosal firewall

2020 Poultry Science 99:1906–1913
https://doi.org/10.1016/j.psj.2019.12.011

INTRODUCTION

The gastrointestinal tract (**GIT**), or "gut", regulates homeostasis of the microbiological, physiological, and physical functions that allows the host to endure infections and other environmental stressors that it encounters (Sansonetti, 2004; Crhanova et al., 2011; Maslowski and Mackay, 2011; Quintero-Fiho et al., 2012). Because the gut has the greatest surface area separating the environmentally exposed lumen and the internal subepithelial tissue, the GIT is constantly exposed to infectious and non-infectious stressors making it an active immune organ containing more resident immune cells than any other organ in the host. The mucosal immune system, a highly regulated network of innate and acquired elements, provides a remarkable ability to respond and modify to these extremely diverse encounters (Honda and Littman, 2016). The development of the different divisions of the immune response has corresponded with the acquisition and maintenance of a symbiotic microbiota. The microbiota trains, stimulates, and functionally adjusts the different features of the immune system (Hooper and Macpherson, 2010; Hooper et al., 2012).

[1]Corresponding author: mike.kogut@ars.usda.gov

The Avian Immune System

The immune system of vertebrates is a multifaceted network of molecules and cells that coordinate responses against infectious agents, toxins, and danger signals while maintaining tolerance to self-antigens. The mechanisms of the host immune response of vertebrates are classically separated into 2 interdependent branches: (1) the "hard-wired" responses encoded by genes in the host's germline and that recognize molecular patterns shared by multiple microbes/dangers that are not in the host—the innate immune response and (2) responses encoded by gene components that somatically rearrange to assemble antigen (Ag)-binding molecules, with specificity for individual, unique foreign structures—the acquired/adaptive immune response.

Innate Immunity The innate immune response is a multilayered set of reactions against pathogenic organisms and environmental insults (Medzhitov, 2007). At the cellular level, the innate immune response is mediated by epithelial cells in mucosal surfaces and phagocytic cells recruited from the blood, including granulocytes, monocytes, and macrophages (Medzhitov and Janeway, 1997). At the molecular level, innate immune cells sense microbes through pattern recognition receptors (**PRR**), which recognize molecular signatures (microbial-associated molecular patterns (**MAMP**)) from microbial cells, such as lipoproteins, carbohydrates, and nucleic acids (Janeway and Medzhitov, 2002; Kumar and Akira, 2011). Pattern recognition receptors also recognize host signature molecules that are indicative of disease and cellular damage—damage-associated molecular patterns (Garg et al., 2010; Krysko et al., 2011). Pathogen recognition results in the activation of cellular defense mechanisms, production of secreted pro-inflammatory cytokines, recruitment of immune cells to the site of infection, and stimulate anti-microbicidal mechanisms, such as the production of reactive oxygen species and antimicrobial peptides (Carpenter and O'Neil, 2007; Lee and Kim, 2007; Underhill, 2007; Takeuchi and Akira, 2010).

Although a certain degree of redundancy exists between signals induced by various PRR, in general, no single PRR is likely to be the sole mediator of activation of the innate immune response. Therefore, a variety of pathogens, each containing different MAMP, can interact with a certain combination of PRR on or in a host cell that trigger specific signal transduction pathways that will induce specific gene expression profiles best suited for the pathogen (Cheng et al., 2006; Gowan et al., 2007; Mogensen, 2009). Hence a combination of toll-like receptor (**TLR**) and non-TLR PRRs will be triggered during infections.

Acquired Immunity The defense mechanisms of acquired immunity depend on the recognition of foreign proteins collectively known as antigens. Adaptive immunity is slower series of reactions primarily mediated by B and T lymphocytes. The initiation of the acquired immune response is based on the recognition of an antigen by a specific receptor expressed on the surface of T or B lymphocytes (Erf, 2004). The antigen specificity of the receptors is encoded by genes that are assembled by somatic rearrangement of a number of gene components to form intact T cell receptor and immunoglobulin B cell receptor genes. The assembly of antigen receptors from a collection of a few hundred germline genes permits the formation of millions of different antigen receptors, each with potentially unique specificity from a different antigen.

Activation of adaptive immunity results in the production of antibodies by B cells, the killing of infected host cells by cytotoxic T cells, and various helper T cell-mediated actions. Most importantly, the end products of an adaptive immune response is the production of memory B and T cells that provide long-term specific protection against subsequent infections with a pathogen bearing the same antigens. Like mammals, chickens have both a humoral and cell-mediated branches of the acquired immune system (Wigley, 2013). The bursa of Fabricius is a unique organ of birds that is essential for B lymphocyte development and humoral immunity (Glick et al., 1956). Embryonic stem cells migrate to the bursa and undergo proliferation that persists for several weeks at hatch. These precursor B cells have already rearranged their immunoglobulin genes prior to entering the bursa; unlike mammals, the chicken has a very limited number of variable genes using a process called gene conversion to create antibody diversity. In gene conversion, the variable heavy and light chains are replaced with upstream pseudogenes (Benatar et al., 1992). The cell-mediated component of the acquired response includes ab and gd T cells and natural killer cells (Chai and Lillehoj, 1988; Guo et al., 2013; Straub et al., 2013). Both CD4 and CD8 T cell subsets are present with Th1 CD4+ cells having a similar function as found in mammals (Gobel et al., 2003; Guo et al., 2013). Further Th2 cells have been shown to function as well (Kaiser et al., 2005).

Intestinal Immunity

Like the systemic immune system, the mucosal immune system is made up of a network of innate and acquired elements. However, unlike the systemic immune system, the intestinal immune system has 2 distinct functions: the ability to respond to pathobionts (potential pathogenic microbes), invasive pathogens, and microbial products while also maintaining a state of tolerance to the diverse and beneficial commensal intestinal microbes (Broom and Kogut, 2018). Both systems working together through innate immune sensing using PRRs on epithelial cells and professional immune cells in the lamina propria (dendritic cells (**DC**) and macrophages), trigger immune pathways resulting in microbial killing and the activation of various acquired immune effector T cells (Th1, Th2, Th17, and Treg) all while keeping the resident microbiota in check without generating an overt inflammatory response.

The intestinal innate defenses are characterized by a "mucosal firewall", a system of barriers that separates

the luminal side of the intestine from the subepithelial tissues (Belkaid and Hand, 2014). The reliability of the mucosal firewall is vital for the interactions between the immune system components and the intestinal contents. The first component of the mucosal firewall is the microbiological barrier where the microbiota lives in or at the upper mucus layer. These commensal bacteria function to provide colonization resistance against pathogen colonization, produce metabolites/components that modulate immune signaling, and promote immune homeostasis (Garrett et al., 2010, Belkaid and Hand, 2014; Belkaid and Harrison, 2017).

The second firewall is the chemical barrier consisting of the mucus overlying the gut epithelium. The mucus regulates contact between the commensal bacteria and the epithelial cells. This division between the epithelium and commensals is achieved by the activity of the mucus produced by goblet cells in the epithelium, anti-microbial peptides released by the epithelial cells, and mucosal IgA produced by DC in the intestine (Belkaid and Harrison, 2017).

The third component of the firewall is the physical barrier provided by the single cell epithelial cell layer. The intestinal epithelium is a single cell layer that assists the absorption of nutrients while providing a physical barrier that prevents both pathogen invasion and extra-intestinal translocation of commensal microbes. Besides being the primary barrier preventing a microbial breach of the intestine, the epithelial cells should also be considered part of the cellular component of the innate immune response possessing PRR for sensing microbial MAMP, but also capable of producing cytokines and chemokines to drive an inflammatory response against pathogen infection.

The final component of the mucosal firewall is the immunological barrier where the professional immune cells (macrophages, DC, and lymphocytes) reside in the lamina propria (Abraham and Medzhitov, 2011). Further innate sensing of microbes is conducted by macrophages and DC that present antigens to T cells resulting in the differentiation and activation of various T cell subsets (Th1, Th2, Th17, or Treg) (Abraham and Medzhitov, 2011). Specialized epithelial cells of the GIT function together with lymphoid, myeloid, and stromal cells to secrete mucus, anti-microbial peptides, IgA, and chemokines that limit direct contact between the epithelium and infectious agents and activate target cells that mediate innate defenses (Medzhitov, 2001; Abreu et al. 2005; Akira et al., 2006; Kawai and Akira, 2009; Mantis and Forbes, 2010). The importance of these epithelial defense mechanisms is highlighted by the ability of enteric pathogens to target these mechanisms to achieve invasion and dissemination (Lu and Walker, 2001; Fischback et al., 2006; Alemka et al., 2012; Awad et al., 2017; Goto et al., 2017). This infiltration of immune cell in lamina propria is inversely correlated with weight gain (Belote et al., 2018), showing that this final component of the mucosa firewall has a metabolic cost for the host that affect animal performance (Kogut and Klasing, 2009).

Microbiota Interactions With the Immune System

The host-microbiota interaction is exceedingly complex that affects the host metabolism, immunity, and health (Marchesi et al., 2016). This crosstalk is mediated by dietary nutrients, host and microbiota metabolites, as well as antimicrobial compounds. Microbiota growth and anatomical location are regulated by the host through production of non-specific antimicrobial peptides such as defensins (Xiao et al., 2006; Bommineni et al., 2014), IgA (Wieland et al., 2004; Lammers et al., 2010; Den Hartog et al., 2016), and miRNAs that regulate bacterial transcripts and bacterial growth (Liu et al., 2016).

The commensal microbes in the intestinal tract sense the local environment to induce biochemical pathways to activate bacterial metabolism that allows them to avoid, alter, or survive host innate immune killing. Further, some microbial-based molecules can promote specific commensal processes that are beneficial to both host and microbe. Similarly, the host detects the microbes through their production of specific molecules or components with unique molecular patterns that lead to the activation of innate and acquired immune responses. Thus, the adaptation of the commensal bacteria (as well as viruses and fungi) living in the intestine of a host has resulted in a mutually beneficial coexistence for both microbiota and host during homeostasis (Kogut, 2013; Kogut et al., 2017; Broom and Kogut, 2018). The interdependent relationship between host and microbiota pointedly influences the host immune response to induce immune tolerance to commensal microbes while also maintaining responsiveness to invading pathogens (Bene et al., 2017; Guo et al., 2017; Shi et al., 2017). Altering the intestinal microbial communities disturbs this immune balance and leads to immune dysregulation and susceptibility to diseases.

Sensing of the microbiota by PRRs generates several mechanisms that promote the host–microbiota relationship while preventing infection by pathogenic organisms. Microbial signals induce pro-inflammatory cytokines such as IL- 23 and IL-1b from macrophages and DCs that then activates IL-17 and IL-22 production by T cells, leading to the production of antimicrobial peptides (AMPs). Dendritic cells can carry microbiota antigens to both or either the Peyer's patches and small lymphoid follicles in the avian intestine, where they drive the differentiation of regulatory T cells (**Tregs**) and Th17 T cells that, in turn, induce the differentiation of IgA-producing plasma B cells that secrete further amounts of IgA.

The microbiota is directly engaged in maintaining the functional innate immunity of the host. The host immune system consistently senses the intestinal microenvironment to determine the metabolic state and colonization status (Levy et al., 2016). In the steady-state, either or both of the metabolites and components of the commensal microbiota are recognized by various

PRRs, including TLRs and NOD-like receptors (NLRs), to regulate intestinal epithelial barrier function, cellular lifespan of phagocytes, and induce secretion of AMPs and IgA (Levy et al., 2016; Blacher et al., 2017). Further, beneficial bacteria ferment dietary fibers to produce small chain fatty acids (**SCFA**) which stimulate the production of anti-inflammatory cytokines (Levy et al., 2016; Blacher et al., 2017) that drives the production of Tregs. Further, the microbiota influences the priming signal of the inflammasome activation that leads to the transcription of tumor necrosis factor-α and IL-6, as well as pro-IL-1β and pro-IL-18. The gut microbiota is involved in maintaining intestinal immune homeostasis by stimulating different arms of the T-cell response. Segmented filamentous bacteria are potent promoters of Th17 cells in the intestine; whereas, polysaccharide A from the commensal *Bacteroides fragilis* stimulates the generation of Tregs (Levy et al., 2017). Alternatively, pattern recognition by TLRs and NLRs can also induce the maintenance of tolerance (Levy et al., 2017).

Lastly, it has become readily apparent that the intestinal immune system can also detect the metabolic state of the microbiota by recognition of microbial metabolites via their PRRs (Blacher et al., 2017; Levy et al., 2017). The microbiota, using several biochemical pathways, metabolizes both diet- and host-derived metabolites that then influence various components of the intestinal immune system. For example, the microbiota converts non-digestible fibers to SCFA that have a number of anti-inflammatory activities (Postler and Ghosh, 2017). The microbiota can degrade dietary tryptophan into indoles, which promotes epithelial cell barrier function (Postler and Ghosh, 2017). Likewise, the microbiota can metabolize dietary arginine to polyamines that inhibit the production of pro-inflammatory cytokines by macrophages (Postler and Ghosh, 2017). The microbiota converts primary host-derived hepatic bile acids to secondary bile acids that inhibit pro-inflammatory cytokine secretion by DCs and macrophages (Thaiss et al. 2014). Besides having a repertoire of metabolite sensing receptors, the host has developed immune signaling pathways (inflammasomes) expressed in various intestinal cell subsets (macrophages, DCs, epithelial cells, and T cells) that recognize microbial-mediated metabolic activity that can stimulate anti-microbial activity involved in stable colonization of the intestine (Levy et al., 2015; Levy et al., 2015; Wang et al., 2015; Birchenough et al., 2016). Therefore, there is intimate cross-talk between the microbiota and the host that is steered by metabolite secretion and immune signaling that has a critical influence in animal health and disease through multiple physiological functions of the host.

Pathogen Interactions With Microbiota and Immune System

The epithelial surfaces of the vertebrate intestine are continuously exposed to pathogenic bacteria, fungi, viruses, and parasites while also associated with the intestinal microbiota. Thus, the intestinal epithelial cells are faced with the unique challenge of directly interacting with enormous numbers of microbes that include both pathogens and commensals. As described above, commensals have evolved methods using metabolites to mediate and establish their symbiotic states in the host. On the other hand, pathogens translocate effector proteins into host eukaryotic cells that facilitate their parasitic existences. Pathogens can affect gut integrity and function (Droleskey et al., 1994) and pose a threat to the immune system (Neish, 2002). The collective gut ecosystem itself may possess pro- and anti-inflammatory determinants with viral, bacterial, and fungal components (lipopolysaccharide [LPS], peptidoglycan [PGN] flagellin, RNA, DNA, and glucans) serving as pro-inflammatory mediators while translocated proteins, such as AvrA (Arsenault et al., 2016) and dampen inflammation. Thus, microbiota are often involved in both a protective role during infections (immune modulation and colonization resistance), but also may act as a reservoir for opportunistic pathogens that can flourish under appropriate conditions. We are only beginning to understand the interactions that connect the host intestinal mucosa, the microbiota, and pathogens.

The microbiota also plays a role in the resolution of an immune response following an infection. Any disruption in the diversity of the beneficial microbiota can lead to the appearance of opportunistic pathogens or trigger the translocation of otherwise non-pathogenic bacteria into the extra-intestinal organs. How the immune system deals with these microbiota shifts in population is understudied in poultry although infection of the host with opportunistic bacteria, such as members of the Enterobacteriaceae have been described (Han et al., 2017; Connerton et al., 2018). Based on the role that microbiota metabolites play in immune development and coordination, severe shifts in the microbiota may also result in alterations in metabolite production and that the metabolome may be measured as a biomarker for the health of the microbiota (Yan et al., 2016). How microbiota-derived metabolites shift and modulate immunity at times of infection is not known.

Viral Infections Perumbakkam et al. (2014) described a relationship between viral infection and microbial composition of the intestinal tract that may influence inflammation and immunosuppression of T and B cells in the chicken. Marek's disease virus (**MDV**) infection altered the core gut flora in the total fecal samples relatively early after infection (2 to 7 D) and in the late phase of viral infection (28 to 35 D) in cecal samples, corresponding well with the life cycle of MDV. The genus Lactobacillus was exclusively present in the infected samples in both total fecal and cecal bird samples. The community colonization of core gut flora was altered by a viral infection, which manifested in the enrichment of several genera during the early and late phases of MDV replication. These investigators further (Perumbakkam et al. (2016) demonstrated a difference in microbiome

community structure and changes in metabolic profiles between MDV-susceptible and resistant lines of chickens.

Avian influenza virus infection in chickens induced a shift in the gut microbiota with an increase in Proteobacterium with an increase in Vampriovibrio, Pseudofalvonifactor, Ruminococcus, and Clostridium cluster XiVb (Yitbarek et al. 2018a, 2018b). The authors speculated that this disruption of the gut microbiota might be a mechanism whereby the virus establishes infection in chickens.

Bacterial Infections As mentioned, earlier, the gut microbiota also plays a central role in the protection of the host from enteric bacterial infection. Nevertheless, many enteric pathogens have developed strategies to outcompete the intestinal community, leading to either or both infections and chronic diseases. A newly hatched chick does not have a maternal microbiota as they are housed separately from the adult hens immediately after hatch in commercial production (Crhanova et al., 2011). Therefore, the GIT of newly hatched chickens is presumed sterile and presents an empty ecological niche that provides easy access for the pathogen to colonize with limited restriction (Crhanova et al., 2011). This factor alone makes young chickens highly susceptible to enteric bacterial infections, such as *Salmonella,* which can result in different degrees of disease spectrum from a subclinical carrier state to a high mortality rate depending on the infecting bacterial serovars and host's susceptibility. These results were further confirmed by other experiments form the same laboratory, although the most changes in microbiota were found when infection occurred in newly hatched chicks when compared with infection of 4- and 16-D old chicks (Juricova et al., 2013). The early exposure of young chickens to *Salmonella* effects on the microbiota composition was further shown by Mons et al, (2015). Microbial diversity was reduced in *S. enteritidis*-infected birds. Disruption of the microbiota community was associated with the expansion of bacteria of the family Enterobacteriaceae early in the post-infection period. Decreases in butyrate-producing bacteria of the Lachnospiraceae family were negatively correlated with a high prevalence of Enterobacteriaceae family, suggesting a competitive interaction between the two bacterial taxa in the gut.

Transcription of pro-inflammatory cytokines negatively correlated with phylum Firmicutes and positively correlated with Proteobacteria (Oakley and Kogut, 2016), with genera of *Escherichia/Shigella, Parasuttterella, Vampirovibrio* being positively correlated with IL6 cytokine expression, while an inverse correlation with Firmicutes (genus Fecalbacterium) was also found. Interestingly, it has previously been found that *Fecalbacterium* is able to secrete metabolites that blocks NF-kβ activation and IL-8 production (Sokol et al., 2008). Further, the genus *Caloramator* was also negatively correlated with IL-6 but positively correlated with the anti-inflammatory TGF-B4 expression (Oakley and Kogut, 2016).

Although the host immune responses have essential roles in preventing *Salmonella enterica* infection, *S. enterica* takes advantage of those responses to overcome gut colonization resistance. *Salmonella enterica* serovar Typhimurium was unable to colonize the intestine without inflammation in the gut because the gut microbiota is thought to outcompete the intruder. However, *S. enterica* serovar Typhimurium was able to invade the host when there was a host inflammatory response in the gut (Stecher et al., 2007; Santos et al., 2009; Winter et al., 2010). Effector molecules secreted by *S. enterica* T3SS and host detection of pathogen-associated molecular patterns, including peptidoglycan, flagellin, and lipopolysaccharide, initiate the host inflammatory responses by inducing the production of pro-inflammatory cytokines, including IL-1β and IL-18. Host inflammation induced by *S. enterica* alters the composition of the gut microflora, which might also create favorable conditions for the growth of *S. enterica.*

Immune gene expression trends were found predominately in the cecum and not the ileum of chickens infected with *Salmonella* or *Campylobacter* with early infection (0 to 7 D of age) associated with increased expression of beta-defensin. The late infection resulted in a decreased IL-4 and IL-10 expression that was associated with modulation in the gut microbial community; whereas interferon-gamma (IFN- γ) increased until day 22 of age. Further, a greater abundance of Proteobacteria was observed during early growth that was later replaced by Firmicutes as the birds aged; therefore, the modulation of commensal bacteria as the bird matures appeared to also affect host immunity.

Connerton et al. (2018) recently described an age-dependent intestinal immune response to a *Campylobacter* infection that was associated with levels of colonization and permanent alterations in the cecal microbiota communities. Connerton et al. (2018) reported that the late infection (> 3 wk of age) resulted in a pro-inflammatory Th17 response with reduction in the abundance of *Clostridium* cluster XIVa. Further, Awad et al, (2016) found that *Campylobacter* colonization resulted in a significant reduction in *Escherichia coli* throughout the intestine, but an increase in *Clostridium* spp. Others have shown that the gut microbiota appears to contribute to *Campylobacter* control and prevent the development of intestinal lesions, although the mechanisms mediating these effects are not known (Han et al., 2017).

Parasite Infections Infection with *Eimeria tenella* suppresses the growth of most bacterial species except members of Enterobacteriaceae in birds with normal intestinal microbiota (Hauck, 2017). Infection with *E. acervulina* reduced the bacterial diversity as well as the homogeneity among chicks in the ceca but not in the ileum (Perez et al., 2011). Mixed infection with *Eimeria acervulina, Eimeria maxima, and Eimeria brunetti* decreased bacterial diversity in the ceca (Stanley et al., 2014). The most affected bacteria, based on pyrosequencing of 16S rDNA, were *Clostridium, Lactobacillus,*

Eubacterium, and *Ruminococcus*. Furthermore, the mixed infection increased the numbers of culturable coliform bacteria and enterobacteria. Interestingly, the infection also significantly decreased the frequency at which the immune-modulating bacterium *Candidatus savagella* was detected (Stanley et al., 2014). In a similar study, Ruminococcaceae groups were reduced, and three unknown *Clostridium* spp. were increased in abundance after infection with these three Eimeria spp. (Wu et al., 2014). Bacteria-free chickens had less severe clinical signs and pathological lesions after infection with *E. tenella* than conventional controls (Visco and Burns, 1972; Bradley and Radhakrishman, 1973). The bacteria seemed to not only aggravate the lesions but also increase the replication of the coccidian. However, there was no difference in oocyst shedding (Visco and Burns, 1972). Further results showed that *E. tenella* infection significantly impacted the abundance of bacteria in the order Enterobacteriaceae (increased) and Bacillales and Lactobacilliales (decreased) which were associated with lesion severity (Macdonald et al., 2017).

Only chickens with natural intestinal microbiota develop typical lesions after infection with *Histomonas meleagridis*, whereas the parasite causes only mild atypical lesions in gnotobiotic chickens with a single species as intestinal microbiota or with the combination of *E. coli* and *Clostridium perfringens* (Springer et al., 1970).

Okulewicz and Złotorzycka (1985) found a lower diversity of the microbiota and a smaller number of bacteria in the intestinal content of commercial hens infected with the nematode, *Ascaridia galli*, than in non-infected hens. Indeed, it has been shown for other nematodes that they are capable of producing various types of antimicrobial molecules (Midha et al., 2017).

CONCLUSIONS AND PERSPECTIVES

The maintenance of intestinal homeostasis is a delicate balance between the host and the microbiome. While the host devotes several physiological mechanisms to compartmentalize the intestinal microbiome, the intestinal immune system needs microbial stimulation for proper development and regulation. Conversely, while individual members of the microbiome can activate specific arms of host mucosal immunity, these host responses prevent inappropriate overgrowth or translocation of members of the microbiota. Additionally, microbiome-driven host responses can also prevent the development of inappropriate inflammation. The end result is physiological homeostasis, where microbial stimulation promotes normal immune function in the intestine, which in turn allows the intestinal microbiome to flourish in the absence of unnecessary inflammation. The role of the gut microbiota in resisting colonization of enteric pathogens, educating and promoting the maturation of the host immune system, and host metabolism underscore the need to understand mechanisms underlying the myriad of supportive roles of the gut microbiota in avian health. Further research in the integration of the microbiota, and their metabolites, as intrinsic regulators of these immune responses, is important to uncover more details regarding the specific features of intestinal microbiota responsible for the differential induction of immune cell populations in the "steady state" and in inflammatory processes that promote the avian health and welfare.

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