The Microbiota Regulates Immunity and Immunologic Diseases in Dogs and Cats

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Animals are obliged to develop a relationship with the microbes that live on their surfaces. The enormous and diverse population of microorganisms living on the skin and within the respiratory and digestive tracts directly influences the development, regulation, and function of the immune system. Conversely, the immune system regulates the composition and behavior of these microbial populations. Immune responses are therefore profoundly influenced by the microbiota.

Body surfaces constitute stable, nutrient-rich ecosystems where microbes thrive. As a result, they are densely populated by bacteria, archea, fungi, and viruses, collectively termed the microbiota. It is estimated that in an animal body at least half of all the cells are microbial. These microbes release a complex mixture of metabolites, vitamins, and nutrients that signal to the host’s immune system and influence the immune response.

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system development, function, and behavior. This is reflected in profound effects on
the development of immunologic and allergic diseases. These microbial effects must
be considered while treating such complex diseases and they provide possible routes
to new innovative treatments.

The body’s surface defenses are faced with the task of coexisting with the
microbiota while simultaneously preventing any invasion of pathogens through
breaks in the epithelial barriers. Nutrients and microbial metabolites are continually
released into the body by the microbiota where they influence immune cell and
inflammatory functions. These products, detected by the cells of the immune
system, ensure that the immune system is prepared to respond promptly to micro-
bial invasion. In fact, the body’s response to the microbiota involves 2 opposing
processes. Stimulation by microbial products serves to activate the immune
system. However, to ensure that inflammation and other immune processes are
not excessive, this stimulatory response must be counterbalanced by regulatory
processes.

For many years, it was believed that the role of the immune system was simply to
ensure exclusion of all invading microbes by distinguishing between self and
not-self and eliminating foreign antigens. It is now known, however, that the immune
system must also determine the degree and nature of the threat posed by the
microbes it encounters and adjust its responses accordingly. It must tolerate the
microbiota and food antigens while simultaneously be highly responsive to invading
pathogens. It must decide, when necessary, whether to mount a cell-mediated or an
antibody-mediated response. This discrimination is determined both by how the
antigens are processed and by signals from the microbiota. The presence of the
microbiota must either be tolerated or ignored if an animal is to remain healthy.
An animal cannot afford to act aggressively toward its own microbiota. The pres-
ence of all these bacterial products has the potential to trigger massive acute
inflammation; however, this inflammation must not happen unless absolutely
required to defend the body.

Therefore, in a normal, healthy animal, 2 opposing responses are in balance. The
body, although not responding aggressively to the microbiota, must be prepared to
respond rapidly and effectively at any time. The body must refrain from overrespond-
ing aggressively to the microbiota while being ready to respond rapidly and effectively
when necessary.

If this equilibrium between the proinflammatory and antiinflammatory processes is
disturbed, this will be reflected in changes in the level of activation of the immune
and inflammatory systems.

HOW THE MICROBIOTA IS ANALYZED

The existence of the microbiota has been known since the invention of the micro-
scope. Its importance, however, has been generally underappreciated until recently.
Advances in technology have allowed for a deeper understanding of the complex
ecosystem of the microbiome and its interactions with the host immune system.

In the past, intestinal bacteria were identified by growing them in culture; however,
this only allowed a small proportion of the highly diverse microbial ecosystem to be
identified. The anaerobic nature and restricted cultural conditions of most gut bacteria
in cats and dogs, as well as limited knowledge of appropriate media for many bacterial
species, prevent culturing them. However, prokaryotes can now be identified and
classified within complex mixtures by sequencing their specific DNA. As a result,
much of the characterization of the gastrointestinal microbiota has focused on the
bacterial composition. Although commensal archaea, fungi, and viruses reside in the gut, their role in the health and disease of the host, although clearly important, remains poorly understood.7–9

There are many molecular tools that can be used to characterize the microbiota. This analysis first requires acquisition of an appropriate intestinal sample (feces, luminal content, or biopsy; swab or scrape in cases of postmortem sample collection) from which DNA or RNA can be extracted. The sample DNA is extracted, isolated, and purified. This DNA can then be identified by amplifying specific genes using universal primers, and quantified using quantitative real-time polymerase chain reaction (qPCR) and fluorescent in situ hybridization (FISH).7

For phylogenetic identification, the 16S ribosomal RNA (rRNA) gene, which encodes the small subunit of rRNA and is thus present in all bacteria, is often targeted.6,7,9 The 16S rRNA gene is found only in prokaryotes and, although highly conserved within a bacterial species, it contains hypervariable regions with specific sequences that can be used to identify the bacterial species.7 These 16S rRNA genes are sequenced using primer sets that amplify 1 of their hypervariable regions. A next-generation sequencing platform, such as the Illumina platform or 454-pyrosequencing, can be used to produce reads of about 75 to 500 base pairs. This is only a small portion of the 16S rRNA gene which, in total, contains approximately 1500 base pairs. Using platforms such as these is more cost-effective than complete genome sequencing and still permits 1 or 2 specific hypervariable regions to be assembled. Other methods of sequencing include shotgun metagenomics and transcriptomics, which through new high-throughput sequencing platforms permits sequencing of the total genomic DNA or mRNA without previously amplifying specific genes.6,7,9 These methods permit determination of a core microbiome, defined as the number and identity of bacteria that are shared among different individuals, and identification of a functional profile of the microbiome.6,7,9 Metabolomics using mass spectrometry to examine the metabolites produced by bacteria can be used to analyze changes in biochemical pathways of the host caused by dysbiosis.6,7,9 Molecular fingerprinting produces a representation of the bacterial community through separation of a mixture of polymerase chain reaction (PCR) amplicons generated by universal primers. Different techniques can be used in molecular fingerprinting, including denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE), and terminal restriction fragment length polymorphism.6 Although these techniques are inexpensive and can be performed rapidly, DGGE and TGGE only allow changes in the predominant bacterial groups to be examined. Quantification of bacterial groups can be done using qPCR and FISH.6

Following sequencing, bioinformatics analysis of the raw DNA data can be performed. The bacterial population is compared with a comprehensive database of known 16S rRNA genes, permitting classification and identification of the microbes present. Duplicates are removed, as are reads that indicate plant material, eukaryotes, and archaea, which suggest the presence of contaminants such as food or host genomic material. The remaining sequences can then be assigned into groups known as operational taxonomic units, which refer to clusters of unknown organisms that are grouped by similarity of their DNA sequence (eg, the 16S rRNA marker gene). Several statistical methods and programs are available to analyze the data obtained. Many of the measures used in microbiome analyses are also commonly used in ecology. For example, evaluation of the bacterial species richness and diversity can be accomplished by determining the alpha and beta diversity (alpha diversity is a measure of diversity within samples, whereas beta diversity measures diversity between samples), which are commonly used in population studies in ecology. The need to analyze
microbial diversity using ecological techniques reinforces the idea that the micro-
biome is a complex ecosystem composed of a diverse array of metabolically active
organisms.

THE NORMAL MICROBIOTA

The development of the immune system in newborn puppies and kittens is driven by
the organisms that colonize the skin and the gastrointestinal and respiratory tracts. These early-life microbial exposures determine how the immune system develops
because germ-free mammals fail to develop their mucosal lymphoid tissues. The
microbiota generates a complex mixture of microbial-associated molecular patterns
that act through enterocyte toll-like receptors (TLRs) to promote the functional devel-
opment of the immune system.10 The intestinal and skin microbiota also contributes to
this process as newborns suckle and are groomed by the mother.11

The Skin

Normal skin harbors trillions of microorganisms on keratinocytes and within seba-
ceous glands and hair follicles.12 It has been estimated that up to a billion bacteria
may live on a square centimeter of human skin. Given the sheltering effects of hairs
or feathers, it is likely that the skin microbiota in domestic animals is even more com-
plex. The skin microbiota of dogs varies greatly between individuals and different skin
sites. For example, there is higher microbial diversity in haired skin compared with
mucocutaneous junctions. The precise composition of the skin microbiota thus de-
pends on location (hairy, wooly, or bald skin; back vs skin in the axilla, groin, or ear)
and the presence of disease such as seborrhea or atopic dermatitis (AD). Grooming
activities have some impact on these microbial populations but their significance is
unclear. The highest microbial diversity on dogs was found in the axilla and the dorsum
of the nose. On average about 300 different bacterial species were identified on the
dorsal canine nose.13 Large populations of Proteobacteria and Oxalobacteraceae pre-
dominate. The feline skin microbiota is also highly diverse. The most common phyla
are Proteobacteria, followed by Bacteroidetes, Firmicutes, Actinobacteria, and Fusob-
bacteria. Major changes in abundance are also observed at different skin sites.14,15

In mice, the skin microbiota influences local inflammatory and T-cell responses. The
microbiota controls the balance between effector and regulatory T (Treg) cells within
skin tissue. They influence keratinocyte production of interleukin (IL)-1 and its effects
on epidermal dendritic cells and thus control local T-cell responses.16,17 Skin bacteria
may activate antigen-specific T cells across the intact epithelium. However, the pres-
ence of Treg cells in neonatal skin mediates tolerance to skin commensal bacteria at a
time when the skin is establishing its microbiota.

The Respiratory Tract

Like all body surfaces, the upper respiratory tract houses a dense and complex micro-
biota. It has been calculated that a human inhales 10^5 organisms per day just breath-
ing outdoor air. Many nasal bacteria are also found on the skin, whereas others are
common environmental bacteria. Deeper in the airways, in the lower respiratory tract,
Neisseria and gram-negative cocci are common.18 The lung is not sterile. Healthy
lungs harbor a microbiota closely related to but much less dense than in the upper res-
piratory tract. The bronchi contain about 2000 bacterial genomes per 1 cm^2. Lung tis-
sues contain between 10 and 100 bacterial cells per 1000 lung cells. These include
aerobes and anaerobes and, like other surfaces, they differ greatly between individu-
als. The predominant phyla are Firmicutes with lesser numbers of Proteobacteria
and Actinobacteria. The organisms found within the mucous layer include not only bacteria but also fungi (yeasts) and viruses such as bacteriophages.19

The Genitourinary System

In adult female animals, lactobacilli and other lactic acid-producing bacteria dominate the healthy cervicovaginal microbiota. The vagina is also lined by squamous epithelial cells rich in glycogen. When these epithelial cells desquamate, the glycogen provides a substrate for the lactobacilli that, in turn, produce large quantities of lactic acid. This reduces the pH to a level that protects the vagina against invasion by many pathogenic bacteria and yeasts. Glycogen storage in the vaginal epithelial cells is stimulated by estrogens and thus occurs only in sexually mature animals.

The Gastrointestinal Tract

The gastrointestinal tract contains an incredibly complex mixture of bacteria, archea, fungi, and viruses. The most obvious of these are trillions of bacteria belonging to hundreds of different species. In mammals, they are dominated by members of 2 phyla: the Firmicutes and the Bacteroidetes, with lesser numbers of Actinobacteria and Proteobacteria, and many minor phyla such as the Fusobacteria and the Verrucomicrobia. Mammals possess about 20,000 protein-encoding genes, whereas their microbiota may collectively possess about 10 million. These diverse genomes enhance an animal’s metabolic potential. They increase its ability to extract energy from plant structural carbohydrates and to obtain essential vitamins. Because of the microbiota, animals can use food sources that would otherwise be unavailable. For example, mice with a conventional microbiota need to eat 30% fewer calories than so-called germ-free mice to maintain their body weight.

Dogs

The dog is more omnivorous than the cat and can digest and absorb a significant amount of carbohydrates. However, it does not depend on microbial fermentation as a major energy source. Nevertheless, a balanced microbiota is essential for canine gastrointestinal health. Each individual dog’s intestinal microbiota is unique and its composition is determined by management, diet, genetics, antibiotic exposure, and environmental factors. The composition of the microbiota also changes along the gastrointestinal tract under the influence of nutrient availability and the local microenvironment.4

The canine stomach has a microbiome dominated by Helicobacter spp. Bacterial counts in the canine duodenum are in a range from $10^2$ to $10^9$ per gram of content. In the colon, the count ranges from $10^8$ to $10^{11}$ colony forming units per gram. The predominant intestinal phyla include the Firmicutes (48%), Bacteroidetes (12%), Proteobacteria (23%), Fusobacteria (17%), and Actinobacteria (1%).20 Clostridiales predominate in the duodenum and jejunum. Fusobacteriales and Bacteroidetes are most abundant in the ileum and colon. Variations result from differences in breed, diet, and age. The Firmicutes consist of mainly gram-positive bacteria, many of which are spore-forming. Important members include Clostridia that may be beneficial or pathogenic. They also include potentially pathogenic Streptococci and Staphylococci. The Actinobacteria are also gram-positive bacteria but with a different G + C content than the Firmicutes. The Bacteroidetes are gram-negative bacteria that ferment indigestible plant carbohydrates to produce short-chain fatty acids (SCFAs). The Proteobacteria include the gram-negative enterobacteria such as Escherichia coli and Klebsiella.
Cats

The intestinal microbiota of the cat, like that of the dog, has evolved with a carnivorous diet. As a result, the cat does not depend on the microbiota to maintain an energy balance. As obligate carnivores, domestic cats rely on a high protein diet. Their predominant phyla include Firmicutes (68%), Proteobacteria (14%), Bacteroidetes (10%), Fusobacteria (5%), and Actinobacteria (4%).

THE MICROBIOTA AND THE IMMUNE SYSTEM

Intestinal Protection

The microbiota protects the body against invasion by pathogens by competing for essential metabolites and nutrients, and by inducing intestinal immune responses. By occupying and exploiting the intestinal niche, commensal bacteria block subsequent colonization by pathogenic bacteria. The microbiota also modifies the intestinal environment by maintaining a low pH and oxygen tension. There is more immune system activity in the intestine than in all other lymphoid tissues combined. It has been estimated that more than 80% of the body’s activated B cells are found in the intestine. Their function is to defend against possible invasion by the microbiota. However, the key to successful accommodation with the intestinal microbiota also depends on the body’s ability to regulate inflammation in the gut wall. This is achieved by maintaining a balance between proinflammatory Th17 cells and antiinflammatory Treg cells.

By studying the changes in the immune system in mice colonized by a single species of bacterium, it has been demonstrated that many different bacteria affect immune function. Many bacterial species have similar, overlapping, or even opposite functions. The enormous diversity and redundancy of the microbiota exerts a complex collective effect on the immune system. Some are potent stimulators of Th17 cells, whereas about a quarter of the bacteria studied stimulate Treg cells. Other bacteria affect innate lymphoid cells (ILCs) and dendritic cells.

Development of Lymphoid Organs

It has long been possible to derive animals by cesarean surgery and raise them within sealed chambers in such a way that they are free of microbes. Compared with conventionally raised animals, these germ-free animals have fewer and smaller Peyer’s patches, smaller mesenteric lymph nodes, and fewer CD4⁺ T cells in the lamina propria of the gut wall. They also have fewer intraepithelial T lymphocytes (IELs) within their intestinal epithelium. These IELs have reduced expression of TLRs and major histocompatibility complex (MHC) class II molecules, as well as reduced cytotoxicity. Germ-free mice have fewer CD4⁺ T cells in the spleen, and fewer and smaller germinal centers, as a result of reduced B-cell numbers. Their production of macrophages and neutrophils is impaired, and their immunoglobulin levels are only about 2% of normal. If exposed abruptly to the external environment, these animals are vulnerable to bacterial invasion.

Mammals have evolved several strategies to generate a diverse antibody repertoire. Thus cattle, sheep, pigs, and rabbits undertake an initial burst of B-cell proliferation with limited diversification in utero. These newly produced cells then migrate to the gut-associated lymphoid tissues where they expand both their numbers and their diversity. This microbial driven B-cell diversification and IgA production depends on the presence of certain bacteria within the microbiota. For example, a combination of Bacteroides fragilis and Bacillus subtilis can induce B-cell development and VDJ diversification in germ-free rabbits. Neither species alone has this effect, suggesting that 2
signals are needed.\textsuperscript{26,27} It is thought that microbial molecules trigger these B-cell responses by binding to their TLRs and activating nuclear factor kappa B (NF-κB) pathways. Alternatively, soluble bacterial superantigens might trigger a polyclonal B-cell response and drive the process by preferentially stimulating the production of B cells expressing certain Vh regions.

**Signals from the Microbiota to the Body**

Bacteria, be they on the skin, respiratory tract, genital tract, or intestine, communicate directly and effectively with their host’s immune system through metabolites and nutrients (\textsuperscript{Fig. 1}). Indeed, this interaction is essential to the proper functioning of the innate and adaptive immune responses.\textsuperscript{28} Alterations or imbalances in the microbiota therefore have profound effects on immune functions.\textsuperscript{29} The proper interaction between the immune system and the microbiota is required for optimal animal health.

Dietary plant fibers contain complex carbohydrates. When digested by Clostridia in the cecum and colon, these carbohydrates generate SCFAs, such as butyrate, propionate, and acetate, which suppress macrophages and promote production of Treg cells. As a result, high-fiber diets play a key role in regulating intestinal inflammation.

Among the intestinal microbiota, some bacteria play a key role in regulating immune responses. For example, Clostridia clusters IV, XIVa, and XVIII specifically induce Treg cells by enterocytes and thus suppress inflammation. The presence of these Clostridial clusters in the colon also results in an increase in the numbers of Treg cells in distant tissues such as the spleen and lung, and they play a role in inhibiting allergic responses.\textsuperscript{30} Thus T cells educated by commensal bacteria emigrate from the gut to remote tissues and determine the body’s T-cell balance.

Enterocytes interact with the intestinal microbiota in a multitude of ways.\textsuperscript{31} They produce peptides that kill or inactivate bacteria and, as a result, shape its composition. They block access of intact antigens to the lamina propria, secrete, and respond to regulatory cytokines and display antigens to dendritic cells. They are vital in ensuring that a balance exists between inflammation and tolerance of the microbiota.\textsuperscript{31} Within the epithelium and the underlying lamina propria are IELs that, on appropriate stimulation by microbiota-induced IL-1 or IL-23, can regulate their differentiation into effector or regulatory cells. By preventing microbial invasion, enterocytes also prevent

\textbf{Fig. 1.} Microbial products and nutrients pass through the intestinal wall and regulate the development and activities of the immune system by maintaining a balance between immunosuppressive Treg cells and proinflammatory Th17 cells. If the microbiota composition changes and dysbiosis results, this balance may be disrupted and animals may become predisposed to developing inflammatory and allergic diseases.
the development of inflammation within the intestinal wall [32]. Antimicrobial peptides within the inner mucous layer keep most of the microbiota from contacting the enterocytes and thus ensure that the microbiota is confined to the intestinal lumen. They not only protect the host from microbial invasion but also from the potentially harmful inflammatory response that would occur if excessive microbial products are absorbed into the body.

Group 3 ILC also regulate the interactions between the microbiota and its host. They produce IL-17 and IL-22 that attract neutrophils and promote the production of antimicrobial peptides in the small intestine. ILC3 cells also activate B cells and induce IgA production.

Although gut microbes are separated by the inner mucous layer and glycocalyx from direct contact with enterocytes, intestinal dendritic cells can extend their dendrites into the intestinal lumen and sample the microbiota. These bacteria may persist within the dendritic cells for several days while they are transported into the mucosa and mesenteric lymph nodes and presented to B cells. In addition, some bacteria are taken up by specialized antigen-capturing M cells, enter the Peyer patches, and become resident within the tissues. Although most of these invading bacteria are killed by macrophages, some are also presented to B cells. The B cells produce IgA, which may bind to bacteria, modify the composition of the microbiota, and block further mucosal penetration.

**Regulatory T Cells**

Intestinal helper T (Th) cell precursors can differentiate into either Treg or Th17 cells on receiving signals from the microbiota. In effect, the microbiota collectively programs the T-cell system to optimize its function and so maximize its survival (bear in mind that the host’s survival is also of importance to the microbiota).

Intestinal Treg cells are a subset of CD4+ helper cells required to maintain the body’s commensal relationship with its microbiota. They produce the suppressive cytokine, IL-10. Treg production occurs in response to signals from both the microbiota and enterocytes. Under stable conditions, the production of Treg cells is favored, whereas that of Th17 cells is suppressed and minimal inflammation occurs within the intestinal wall. In the absence of Treg cells, uncontrolled effector T cells will respond to microbial antigens and trigger inflammation. Mucosal inflammation is therefore actively suppressed by the production of large numbers of IL-10–producing Tregs. IL-10-deficient mice develop chronic unremitting colitis driven by IL-23 and the Th17 pathway.

However, it is also clear that this tolerance can only go so far. Should a potential pathogen seek to invade the body from the intestine, then the immune system must be prepared to act aggressively to prevent this. This is mediated by proinflammatory Th17 cells.

**T Helper 17 Cells**

Th17 cells are a subset of CD4+ helper cells that promote inflammation. Under the influence of IL-23, they produce the proinflammatory cytokines IL-17 and IL-22. Like Treg cells, the development of Th17 cells is regulated by signals from the microbiota and from enterocytes. Th17 cell development is specifically stimulated by the attachment of segmented filamentous bacteria (SFBs) to enterocytes. Enterocytes can sense this tight attachment and stimulate IL-23 production by macrophages, leading to production of IL-17 and IL-22.

SFBs are unique spore-forming, long filamentous, gram-positive anaerobic commensals found in the small intestine of mammals and birds (although they have yet
to be reported in dogs and cats. SFBs stimulate the upregulation of host innate defense genes and inflammatory cytokines. They attach very strongly to the enterocytes of the ileum where they can be sampled by dendritic cells (most other commensal bacteria remain within the mucous layers). SFBs induce the development of germinal centers in Peyer’s patches and other intestinal lymphoid organs, and increase production of IgA and Th17 cells.

**DYSBIOSIS**

If the intestinal microbiota becomes unstable or imbalanced, dysbiosis occurs. It is possible to target specific pathogenic bacterial groups in real time, determine a dysbiosis index using qPCR, and then determine levels of functional microbial metabolites, including bile acids and SCFAs. The Dysbiosis Index developed by the Texas A&M College of Veterinary Medicine Gastrointestinal Laboratory is a rapid PCR-based assay that can be used to quantify 8 different bacterial groups commonly present in the gastrointestinal tract of dogs and cats and express them as a single value that can help indicate the level of dysbiosis. It can also predict normal or abnormal conversion of fecal bile acids, which are affected by alterations in the gastrointestinal microbiota. Dogs show an increase in the Dysbiosis Index in instances of chronic enteropathy, exocrine pancreatic insufficiency, or antibiotic-induced dysbiosis. In many cases, it is not always clear whether this dysbiosis is the cause or the effect of the disease.

The loss of commensal bacterial microbiota has been linked to metabolic changes. Dysbiosis is a cause of equine laminitis and bovine ruminal acidosis, and has been implicated in the development of diseases such as canine chronic enteropathy and inflammatory bowel diseases. Antibiotic treatment is an important cause of dysbiosis because it can radically alter the composition of the intestinal microbiota and increase the risk of developing infections with organisms such as *Clostridium difficile* or overgrowth with other unwanted pathogens. Antibiotics can also alter the composition of the microbiota, resulting in an increased risk of obesity (obese individuals have more Firmicutes and fewer *Bacteroides* than lean ones). Perhaps the most significant dysbiosis is that which leads to the development of allergies.

**The Hygiene Hypothesis**

The prevalence of allergic disease has increased significantly in Western societies over the past 50 years. Although most obvious in humans, this has also affected their pets. It is likely that this increase is, at least partially, a result of changes in their microbiota. The hygiene hypothesis suggests that alterations in Western diets, environmental cleanliness, an urban lifestyle, and overuse of antibiotics together cause long-term changes in the intestinal microbiota. Given the close association between pets and humans, it is unsurprising that the microbiota of pets has come to resemble that of humans. Dysbiosis in humans may well be reflected in their pets. Because the intestinal microbiota influences the Th1-Th2 balance, it is suggested that, as a result of this loss of diversity, Th2 responses come to predominate. Thus the composition of the intestinal microbiota exerts a significant influence on allergy development.

The hygiene hypothesis has received support from studies on piglets. Major differences can be found in their gut microbiota depending on the environment in which piglets are raised. These differences also influence the expression of immune system genes. For example, pigs raised in a very clean environment have reduced microbial diversity and express more genes involved in inflammation.
Conversely, outdoor pigs with a diverse microbiota express more genes linked to T-cell function. Similar effects have been observed in rodents. Germ-free mice have high serum IgE levels in early life. These can be greatly reduced by bacterial colonization, suggesting that the microbiota regulate IgE production. If low doses of vancomycin are fed to neonatal mice, the diversity of their gut microbiota is reduced, their Treg numbers are reduced, and they suffer from increased severity of allergic lung disease. Adult mice treated with oral antibiotics have increased IgE levels and blood basophil numbers. They too have increased airway inflammation following allergen challenge.

Conversely, an appropriately balanced microbiota generates antiinflammatory molecules such as SCFAs, polysaccharide A, and peptidoglycans. SCFAs, such as formate, acetate, butyrate, and succinate and glycans, are produced in abundance in high-fiber diets. Human populations that consume large amounts of fiber have a lower prevalence of colitis and inflammatory disease. Among the SCFAs, butyrate has potent antiinflammatory properties and inhibits proinflammatory responses by intestinal macrophages. Butyric acid acts on macrophages and prevents epigenetic changes by inhibiting histone deacetylases. Butyrate also increases barrier functions by stimulating enterocytes and increasing transcription of mucin genes, goblet cell differentiation, and mucus production. It can also stimulate some bovine neutrophil functions. Therefore, a balanced and healthy microbiota suppresses inflammation by stimulating Treg activity.

**Intestinal Diseases**

Intestinal dysbiosis is often associated with disease development. Chronic enteropathies are a diverse group of diseases that result from a combination of genetic, microbial, nutritional, allergic, and environmental factors. Some resemble inflammatory bowel diseases in humans (Crohn’s disease and ulcerative colitis) but most probably have a different pathogenesis. Some result from dysbiosis in the intestinal microbiota. As pointed out, commensal bacteria within the intestine suppress inflammation by generating IL-10–secreting Treg cells. If these control mechanisms fail and the animal responds aggressively to its commensals by increasing Th17 responses, severe inflammation may result.

Canine chronic enteropathies are characterized by persistent or recurrent gastrointestinal inflammation. These usually present with a history of chronic vomiting, diarrhea, or weight loss. There is a breed predisposition in Weimaraners, Rottweilers, German shepherds, border collies, and boxers. The most common form of enteropathy is a lymphocytic-plasmacytic enteritis. Affected dogs show an increase in Proteobacteria, especially *E coli* or *Pseudomonas*, and a decrease in Firmicutes and Bacteroidetes. Other changes, such as increased bacterial adherence to the mucosa, reduced bacterial diversity, and overgrowth of other bacteria, may all contribute to the inflammation.

Many cases are associated with an increase in T cell and IgA+ plasma cells in the small intestine. Some dogs with chronic enteropathy have reduced IgA levels in feces, duodenum, and blood.

The affected small intestine shows increased messenger RNA for IL-12, IFN-γ, tumor necrosis factor (TNF)-α, and transforming growth factor (TGF)-β. In dogs, cases have been associated with altered expression or dysregulation of TLR-2, TLR-4, and TLR-5. Polymorphisms in TLR-4 and TLR-5 have also been associated with disease susceptibility. Thus it has been suggested that excessive TLR activation might increase IL-1β levels. If this is accompanied by decreased production of its IL-1 receptor antagonist (IL-1RA) then acute inflammation may result. The IL-1RA to IL-1 ratio in
affected dogs is negatively correlated with disease severity. There is no evidence for changes in Th17 cell activity in these diseases in dogs. Likewise, there is no evidence of a Th1-Th2 imbalance in either dogs or cats.

In about 50% of canine cases, feeding a hypoallergenic or a novel antigen diet may result in rapid clinical improvement within a few days and suggests that some forms of enteropathy result from food hypersensitivities. Other forms may result from enteric infections and may respond well to antibiotic therapy. Drugs commonly used include oxytetracycline, metronidazole, and tylosin. These responsive dogs include young large-breed animals and German shepherds. The effects of antibiotic therapy may, however, be short-lived.

Some cases are immunologically mediated and dogs may respond well to glucocorticoids, such as prednisolone, and the immunosuppressive drugs, such as cyclosporine or azathioprine. Unfortunately, the results of many clinical trials have been mixed and confusing, so long-term control remains difficult.

Histiocytic ulcerative colitis in boxers is a severe form of inflammatory bowel disease. The lesions are characterized by the presence of large macrophages that stain intensely with periodic acid–Schiff stain. It has been suggested that this colitis is triggered by an unidentified infectious agent because it somewhat resembles Johne’s disease. The lesions also show accumulations of plasma cells, macrophages, and granulocytes.

Immunoproliferative enteropathy of Basenjis is an inherited autosomal disease that presents as gastric mucosal hypertrophy with lymphoid cell infiltration and ulceration. The whole small intestine may show villous blunting, crypt elongation, and infiltration of the mucosa with lymphocytes, plasma cells, and some neutrophils. Dogs show a polyclonal increase in serum IgA. The disease may be controlled by high doses of corticosteroids.

Protein-losing enteropathy of soft-coated Wheaten terriers is also an inherited disease. Histologic examination of dogs with this condition shows an inflammatory bowel disease. The cellular infiltrates are mainly lymphocytes and plasma cells, but neutrophils and eosinophils are often present. This disease may result from food hypersensitivity, possibly to wheat gluten.

Gluten-sensitive enteropathy of Irish setters is an autosomal recessive small intestinal disease also caused by exposure to wheat gluten. As with the diseases described previously, affected small intestine is infiltrated with lymphocytes and other inflammatory cells. The mucosa shows increased numbers of CD4+ cells and decreased CD8+ T-cell numbers. Affected dogs may also have elevated serum IgA levels.

Lymphocytic-plasmacytic enteritis has also been described in cats, horses, and a cow.

Respiratory Disease

The airway microbiota plays a role in resistance to respiratory infections and development of asthma and chronic obstructive pulmonary disease. Thus, in the absence of a microbiota, the airways are prone to mount exaggerated Th2 responses. The presence of the microbiota induces Treg activity. This probably explains the protective effects of inhaled microbial antigens on the development of allergies in children. Dietary fiber also has a protective effect on allergic airway inflammation in mice as a result of increased levels of SCFAs. The intestinal microbiota also regulates pulmonary adaptive responses. Thus SFBs in the intestine regulate pulmonary immunity to bacteria and fungi. Conversely, influenza infection in the lungs generates type I interferons that, in turn, induce intestinal dysbiosis, such as depletion of obligate anaerobic bacteria and an increase in Proteobacteria.
Atopic Dermatitis

The intestinal and skin surfaces have much in common and interact extensively. The intestinal microbiota with its huge numbers of metabolites may well induce cutaneous changes. The most significant mechanism is through alterations in the Th17-Treg balance, whereas other effects may be mediated by retinoic acid or vitamin A and vitamin D and neuroendocrine interactions.56,57

AD is therefore associated with dysbiosis both in the skin and the intestine.17,58 The skin microbiome seems to influence skin barrier function.59 Staphylococcal colonization correlates with changes in skin barrier function; when the infection was treated and normal microbial diversity restored, normal function was also restored.59

Intestinal dysbiosis also affects the development of AD and manipulation of the intestinal microbiota may assist in the treatment of this disease. Obviously, food allergies may be directly involved in the pathogenesis of atopic skin disease. However, changes in the gut microbiota also influence the level of allergic and inflammatory responses through changes in the Th17-Treg balance. The intestinal microbiota differs from normal in humans with AD,60 and systemic antibiotic treatment increases the risk of AD in humans.61 Early exposure to some probiotics can have both short-term and long-term effects on dogs with AD.62–64

Autoimmunity

Because the gut microbiota influences the development of immunologic tolerance, dysbiosis may also affect the development of autoimmune disease.55,66 For example, nonobese diabetic (NOD) mice develop spontaneous insulin-dependent diabetes mellitus associated with infiltration of the pancreatic islets by lymphocytes. Their disease resembles human type 1 diabetes mellitus. Its development is influenced by their microbiota. Thus conventional NOD mice that lack MyD88 protein (MyD88 is an adaptor molecule for the TLRs) do not develop diabetes, whereas totally germ-free MyD88−/− NOD mice do. If commensal bacteria are given to these germ-free mice, their diabetes is less severe. Somehow, the interaction of the intestinal microbiota with the immune system influences the predisposition of these mice to develop diabetes.

Alterations in the intestinal microbiota also influence autoimmune diseases such as rheumatoid arthritis, ankylosing spondylitis, insulin-dependent diabetes mellitus, and experimental autoimmune encephalitis.67 In some mouse arthritis models, changes in the gut microbiota due to antibiotic treatment can exacerbate the disease. Antinuclear antibody production in mice is influenced by the microbiota, especially by increased colonization with SFB.

SUMMARY

The presence of huge numbers of microbes on body surfaces provides a rich source of signals to the immune system. Given that animals have evolved in the presence of the microbiota, it is unsurprising that the immune system has come to depend on these signals and that changes in the microbiota will provoke changes in these signals that result in alterations in immune function. The mutualistic partnership has evolved to conserve microbial signaling and immune response pathways to ensure an animal’s survival in a world dominated by microbes.51,68 Given the close interactions between the intestine, the respiratory tract, and the skin, it is to be expected that dysbiosis may affect all 3 surfaces or systems. The growing body of data on this subject will likely provide a fruitful source of ideas regarding the prevention and treatment of a multitude of immunologic diseases in domestic mammals.
REFERENCES


