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Review

Protective and pro-inflammatory roles of intestinal bacteria

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ABSTRACT

The intestinal mucosal surface in all vertebrates is exposed to enormous numbers of microorganisms that include bacteria, archaea, fungi and viruses. Coexistence of the host with the gut microbiota represents an active and mutually beneficial relationship that helps to shape the mucosal and systemic immune systems of both mammals and teleosts (ray-finned fish). Due to the potential for enteric microorganisms to invade intestinal tissue and induce local and/or systemic inflammation, the mucosal immune system has developed a number of protective mechanisms that allow the host to mount an appropriate immune response to invading bacteria, while limiting bystander tissue injury associated with these immune responses. Failure to properly regulate mucosal immunity is thought to be responsible for the development of chronic intestinal inflammation. The objective of this review is to present our current understanding of the role that intestinal bacteria play in vertebrate health and disease. While our primary focus will be humans and mice, we also present the new and exciting comparative studies being performed in zebrafish to model host–microbe interactions.

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1. Introduction

The healthy human body contains 10 times more microbial cells than human cells! This proclamation has been repeated many times over the past several years in both the scientific literature, as well as the lay press [169]. Although this declaration has been presented as a scientific fact over the past decade, it may not be entirely accurate in view of a recent reexamination of the published data [169]. Most reviews that focus on host–microbe interactions begin with the statement that the healthy human intestine contains approximately 100 trillion (10^{14}) microbes [55,102,176,178,211]. Furthermore, many of these publications state, without reference, that the total number of human cells in the body approximates 10 trillion (10^{13}) cells [169,178]. However, this 10-fold excess of microbial to human cells may need to be reevaluated based upon more recent work that has been largely overlooked during the past few years. For example, the statement that the intestinal tract contains 10^{14} microbial cells is based upon a 44 year-old report that provides little by way of direct quantitative data for this fecal

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bacterial estimate [108]. Using more sophisticated technology, Suau et al. have determined that the numbers of bacteria that reside within the healthy human intestinal tract range from 3×10^{13} to 40×10^{13} (30–400 trillion) [194]. The assertion that humans contain 10^{13} body cells is based upon one sentence from a 46 year-old book that provides no experimental data nor references for this estimate [37]. A recent study by Bianconi et al. using systematic quantification of cell numbers in different tissues reports that humans contain, on average, 30–40 trillion body cells [12]. These newer data would suggest a more realistic ratio of microbial to human cells that range from 1:1 to 10:1.

While these more quantitative estimates are, in some cases, quite different from what has been repeatedly stated in scientific and lay publications, they confirm that the gut is home to enormous numbers of bacteria. The large majority (>90%) of intestinal bacteria in the human intestinal tract belongs to the phyla Bacteroidetes and Firmicutes. However, substantial numbers of bacteria belonging to phyla Proteobacteria, Actinobacteria, Fusobacteria, Verrucomicrobia and Cyanobacteria are also observed [38,40,44,102,106,176] (Fig. 1). In addition to the enormous population of bacteria, the human gut has also been estimated to contain more than a quadrillion (10^{15}) viruses and bacteriophages, as well as substantial numbers of archaea and fungi [40,55,62,102,106,143,199]. Most of the detailed characterization and bioinformatic analyses of the intestinal microbiota have been performed using human stool and/or mucosal tissue. However, mice have also been extensively used to define the importance of host genetics, microbiota and the immune system in homeostasis and disease [28,141]. The use of mice provide investigators with a small animal model to assess, in a well-controlled environment, the complex host–microbe interactions that occur *in vivo*. While mice and humans share two major phyla (Bacteroidetes and Firmicutes) and approximately 80 different genera [141,168], major differences exist among bacterial species in these two mammals [141,168].

Valuable information has been generated using gnotobiotic and fully colonized mice to assess host–microbiome interactions. However, these studies are limited by the length of time and the high cost associated with the generation of large numbers of genetically-manipulated animals required to yield statistically-powered *in vivo* studies. In an attempt to shorten the time and cost of new discoveries, investigators have begun to use other vertebrates to model these interactions in healthy and inflamed intestine. For example, zebrafish *(Danio rerio)* have become increasingly popular for these types of studies given the similarity of their intestinal tract to that of mammals [57,208]. Although major differences exist between teleost and mammal microbiota, zebrafish share many of the major microbial communities that have been identified in rodents and humans [160,161,180,195,198]. Investigators have shown that similar to humans and mice, the teleost gut contains large numbers of Proteobacteria, Firmicutes and Bacteroidetes [30,166,195] (Fig. 1). The use of teleosts offer a number of advantages over mice and other rodents due to the relatively low cost to produce and maintain large numbers of larvae and adults, their accelerated development, and their transparent skin that allows for detailed and noninvasive imaging studies [161,216]. Another major advantage of zebrafish is their amenability to produce forward and reverse genetic manipulations [216]. Furthermore, because these vertebrates live in an aqueous environment, the delivery of different chemicals/small molecules, therapeutic agents or microbiota to germ-free or fully colonized zebrafish is relatively a straight forward process [19,47,64,160,161,198].

The continuous exposure of the vertebrate intestine to such large and diverse populations of microorganisms in close proximity to a tissue that contains large numbers of immune cells, makes the gut the largest and most complex component of the immune system. The coexistence of vertebrates with their gut microbiota is a dynamic and mutually beneficial relationship that plays an important role in the well-being of the host [29]. However, the close proximity of potentially harmful/pathogenic microorganisms has forced the intestinal immune system to develop a number of different immune mechanisms to eliminate invading microbes, while suppressing the bystander tissue injury associated with these innate and adaptive immune responses. Failure to properly regulate these protective immune responses may induce chronic inflammatory responses that are thought to be critical immunopathological mechanisms responsible for the development of human inflammatory bowel diseases (IBD; Crohn's disease, ulcerative colitis). These idiopathic inflammatory diseases affect primarily the small and/or large bowel and are characterized by the infiltration of large numbers of inflammatory leukocytes (*e.g.*, neutrophils, monocytes, and lymphocytes) into the intestinal lamina propria (LP) where they directly or indirectly promote inflammation with tissue injury, loss of goblet cells, fibrosis, erosions and ulcerations. Although the etiology of IBD remains to be defined, it is becoming increasingly appreciated that chronic intestinal inflammation may result from a complex interaction among genetic, immune and microbial factors [73,99,214]. Based upon a large body of experimental and clinical evidence generated over the past 20 years, investigators hypothesize that chronic gut inflammation results from a dysregulated immune response to components of the normal gut flora in genetically-susceptible individuals [31,79,95]. Although mouse models of IBD have been used for more than 20 years and have been instrumental in defining many of the major immunopathological mechanisms responsible for inflammatory tissue injury in these models, progress as been slow for reasons outlined above [95]. Thus, several groups of investigators have turned to the use of zebrafish to model IBD (see below) [19,47,63,64,216]. The objective of this review is to present our current understanding of the role that the intestinal microbiota plays in vertebrate intestinal health and inflammation. While our primary focus will be humans and mice, we also present the new and exciting comparative studies being performed in zebrafish to model host–microbe interactions.

2. Development of bacterial communities within the intestinal tract

The colonization and development of the intestinal microbiota in all vertebrates is crucial for the generation of a fully functional immune system, production of essential nutrients and vitamins, and metabolism of xenobiotics. While it has been assumed that the development of a stable microbiota in the mammalian gut begins at birth, since *in utero* the fetus has been thought to be germ-free, more recent reports suggest that this may not be the case as bacteria have been isolated from meconium, umbilical cord and amniotic fluid obtained from healthy pregnancies [81]. Nevertheless, the development of a newborn's microbiota begins following birth *via* the colonization of the infant's intestinal tract with bacteria associated with the mother's skin, vagina, feces, and breast milk [102,117,190]. During the first three months of life, *Bifidobacterium* and *Lactobacillus* colonize the intestinal tract in mammals due to the ingestion of breast milk [97,190]. Early on in the infant's life, the microbial communities are highly variable and relatively unstable when compared to the adult microbiota which has much greater complexity and phylogenetic diversity [97,149]. It is thought that the stabilization/maturation of the microbiota occurs at approximately 2–3 years of age and that the microbiota can be prepared with genes for the metabolism of food that is not yet being consumed by the infant (*i.e.*, plant polysaccharide metabolism) [6,97,149,217]. Koenig et al., has shown that the assembly of the microbial communities early in life is not random, but instead, occurs by way of specific bacterial successions due to different life