

# Commensal Microbiota: Powerful Immunological Tools for Gut Homeostasis

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In recent years, several important findings in the fields of gut microbiology and immunology have emerged. An increasing number of studies have investigated the characteristics of commensal microbiota and how their symbiotic relationship with the host can be beneficial for gut health. It is becoming evident that gut microbiota and the intestinal immune system must achieve a complex and delicate balance in order to fight pathogenic bacteria and to protect those that contribute to intestinal

homeostasis and functions. Although microbiota diversity is not fully understood, new evidence suggests that commensal colonization is dependent on dietary, environmental, host genetic and immunological factors. In addition, the recent characterization of the structure, function, and diversity of the healthy human microbiome will be extremely useful for further studies focused on clarifying the immunological and physiological roles of commensal microbiota.

## INTRODUCTION

Intestinal homeostasis is mediated by cross-talk among intestinal resident cells, commensal microbiota, local immune cells, and metabolites. Unfavorable alterations to these interactions are thought to be responsible for the development of inflammatory bowel disease (IBD). IBD includes two major disorders: Crohn's disease (CD) and ulcerative colitis (UC). While CD can affect the entire gastrointestinal tract, especially the ileum, UC results in an inflammation of only the large intestine or colon. Although it is not easy to define the exact etiology of IBD, at least three different causes have been considered to play a major role: genetic predisposition, environmental factors (including the commensal microbiota and diet), and the immune system.

Recent genetic studies on IBD patients have shown around 100 different loci that could participate in the development of IBD. Polymorphisms of genes encoding for IL-23R, NOD2, beta defensin-2, and IRGM are considered to be most commonly associated with the development of inflammatory bowel disorders (Fellermann et al., 2006). Although mutations in these genes, which regulate primarily intestinal barrier functions and mucosal immune responses, have been found to modulate IBD susceptibility, they cannot be employed as predictors of disease.

Increasingly, findings underline the essential role of commensal microbiota in the maintenance of gut homeostasis for at least three major reasons. First, all microbiota deliver trophic and mechanical signals for conducting essential metabolic processes, including bond breaking, nutrient release, and vitamin production, and for enhancing barrier epithelial functions (Sansonetti & Medzhitov, 2009). Second, they prevent colonization of pathogenic opportunistic microorganisms. Third, they actively stimulate the immune system by enhancing the body's defense against pathogens and by maintaining peripheral immune tolerance against all the potential antigens present on the lumen (Mazmanian, Round, & Kasper, 2008). All these microbiota

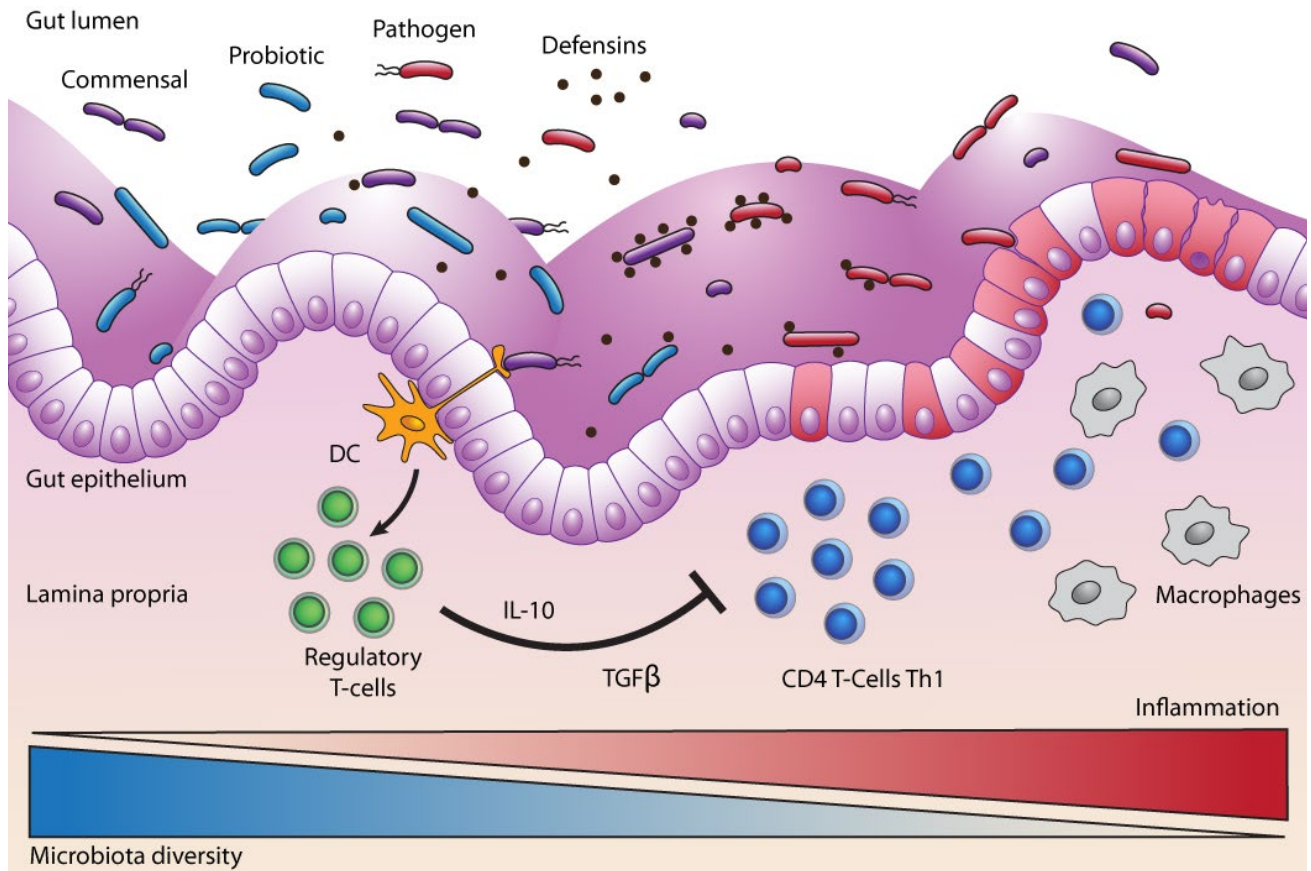
functions occur only through constant mutualism with the intestinal immune and nonimmune cells.

This review aims to discuss and summarize some of the characteristics and functions of the living organisms that reside in the gut in order to underscore their important roles not only in microbiology and nutrition but also in immunology.

## THE COMMENSAL MICROBIOTA

The gut is an organ populated by a complex variety of bacterial communities whose composition determines the activation of both innate and adaptive immune systems (Figure 1). The mammalian gut is the largest surface of our bodies in contact with a bacterial environment. The amount of resident microbiota increases from jejunum through each subsequent part of the gut, reaching up to  $10^{12}$  per gram in the feces. This bacterial community includes more than 1,000 species, and the majority of them are obligate anaerobic organisms (Floch, 2011). Presently, compared to the traditional culture-dependent techniques, new methods, such as genetic screening of 16S rRNA gene and analysis of T cell receptors (Lathrop et al., 2011), have been shown to be more efficient in detecting gut microbiota.

In an adult, most of the gut microbiota belong to two phyla: Firmicutes (Gram-positive anaerobes) and Bacteroidetes (Gram-negative anaerobes), and, to a lesser extent, to the smaller groups of Proteobacteria and Actinobacteria. Indeed, a recent study (Floch, 2011), performed by the National Institutes of Health (NIH) Human Microbiome Project along with the European MetaHIT consortium, demonstrated that, despite the highly complex composition of the commensal microbiota, the gut microbiome is composed of only three major enterotypes irrespective of host age, gender, and body mass index (Arumugam et al., 2011). Therefore, these data suggest that in our guts there is a "limited number of well-balanced host-microbial symbiotic states" (Arumugam et al., 2011). The authors observed that microbiota composition can significantly change in individuals who are developing disorders such as IBD and



**Figure 1 | A wide diversity in intestinal commensal bacteria promotes intestinal health.** The gut is populated by a large community of microorganisms, called “commensal bacteria” or “intestinal microbiota,” that maintain gut health by increasing digestive efficiency, preventing inflammatory processes, modulating immune response, and precluding the entry of pathogens into the lamina propria. This community is numerous, reaching up to  $10^{12}$  bacteria in the colon, and extremely heterogeneous, containing more than 1,000 different species. In a healthy intestine (left half of the figure) the level of inflammation is very low. Commensal bacteria can be sensed by dendritic cells or epithelial receptors. This generates an expansion and activation of regulatory T cells that produce anti-inflammatory cytokines such as IL-10 and TGF- $\beta$ . Those cytokines inhibit the activity of pathogenic CD4T-Cells Th1 and macrophages and promote the release, into the lumen, of anti-inflammatory products such as defensins or IgA, which prevent pathogen bacteria entry. Additional help for the maintenance of the gut’s immune balance can be obtained through the introduction of probiotics. Probiotics are known to mimic commensal bacterial and to stimulate the gut’s immune system, although their exact role is still under investigation. By contrast, a diet rich in fats and sugars, antibiotic treatments, or other environmental factors can strongly reduce microbiota diversity and therefore compromise gut health and encourage inflammation (right half of the figure).

obesity (Arumugam et al., 2011).

Colonization of the intestine begins at birth, either from the immediate exposure to the mother’s vaginal microorganisms or later from feeding. *Bifidobacteria*, belonging to the Actinobacteria phylum, and *Lactobacillus*, a component of the Firmicutes phylum, are established early after natural delivery; however, these species are often late arrivals in the case of Caesarian delivery (Floch, 2011). It has been reported that in breast-fed infants, *Bifidobacteria* are the predominant species; conversely, in formula-fed infants, *Bacteroides*, belonging to the Bacteroidetes phylum, have been found to predominate. As soon as children switch to regular food, the flora assumes the composition and complexity of that of an adult (Floch, 2011).

#### DIETARY IMPACT ON GUT MICROBIOTA

Many studies have clearly demonstrated that dietary habits

are one of the major factors that modulate the gut microbiota (Turnbaugh et al., 2009; De Filippo et al., 2010). In particular, a recent study comparing the gut microbiota composition of children living in a rural African village to that of those living in an industrialized area in Italy demonstrated a remarkable connection between bacterial composition and diet (De Filippo et al., 2010). In the studies described in this elegant paper, the authors found that children fed with a traditional rural African diet, which is low in fat and animal proteins but rich in starch, fibers, and plant polysaccharides, had higher concentrations of Actinobacteria and Bacteroidetes, whereas children fed with a diet richer in sugar, animal fat, and calories (a diet typical of industrialized countries) have a predominance of Firmicutes and Proteobacteria (De Filippo et al., 2010). Another study reports that the ratio of Firmicutes to Bacteroidetes (F/B) increases in obese humans (Ley,

Turnbaugh, Klein, & Gordon, 2006). However, it is still controversial whether there is a correlation between F/B ratio and obesity (Ley et al., 2006; Arumugam et al., 2011) and whether this ratio can be interpreted as a potential obesity predisposition factor.

Dietary fibers contain xylan, cellulose, xylose, and carboxymethylcellulose, which can be converted into short-chain fatty acids (SCFAs) (Flint, Bayer, Rincon, Lamed, & White, 2008) and have been proven to have a protective role against gut inflammation (Scheppach & Weiler, 2004). De Filippo et al. (2010) found that children from rural Africa with diets rich in plant polysaccharides and low in sugar have abundant SCFA in their feces. This indicates that those dietary conditions could specifically select SCFA-producing bacteria (De Filippo et al., 2010; Maslowski & Mackay, 2011), which might prevent the establishment of potentially pathogenic intestinal microorganisms (Hermes et al., 2009). Ultimately, mice that are raised in the absence of microbiota (under germ-free conditions) have an impaired concentration of SCFA (Høverstad & Midtvedt, 1986), and succumb to inflammatory immune disorders (Maslowski et al., 2009; Chervonsky, 2010). These data suggest that diet not only can contribute to the maintenance of a healthier microbial composition, but also can modulate inflammatory responses.

#### THE "HYGIENE HYPOTHESIS"

Commensal biodiversity in the gut can be considered an essential marker for healthy individuals. This microbiota biodiversity can be strongly altered and damaged through ingestion of drugs (e.g., antibiotics, vaccines), during clinical treatments, by improving sanitation, by food composition, and by other environmental factors. Limited contact with microorganisms from the external environment defines what has been called the "hygiene hypothesis" (Strachan, 2000). This term refers to the increasingly common phenomenon, occurring especially in Western countries, of the use of extreme cleaning methods, including excessive hand- or food-washing and intensive sterilization techniques, that drastically reduce the chance for our bodies to encounter microorganisms. While treatments are considered effective for preventing contact with pathogens and toxins, from another perspective they limit contact with microorganisms that could beneficially stimulate components of the innate and adaptive immune systems.

The innate immune system represents the first line of defense for our bodies, especially against infections, but it does not provide a specific or long-lasting immune reaction. Therefore, a second type of defense system, called an "adaptive immune system," is needed to recognize specific antigens and to stimulate more-robust and long-lasting immune processes that can also be remembered throughout life. One of the most important cell types of the adaptive immune system is T cells. On their surfaces, T cells express receptors ("T cell receptors," or TCRs) that bind to specific antigens presented by a group of molecules called a "major histocompatibility complex" expressed on anti-

gen-presenting cells. Classically, an individual's TCR specificities are defined through the self/nonself discrimination processes during thymus development. Inadequate microbial stimulation, especially during childhood, is believed to impair inflammatory T helper 1 (Th1) responses, with a resulting predominance of T helper 2 (Th2)-mediated cytokines such as IL-4, IL-5, and IL-13, which are known to be implicated in the development of allergies (Strachan, 1989; Maslowski & Mackay, 2011). Diseases such as asthma and allergies are extremely rare in several rural African areas (Maslowski & Mackay, 2011). Further, genetic screening of the commensal microbiota has demonstrated that the reduction of biodiversity is also associated with inflammatory conditions such as diabetes and obesity (Arumugam et al., 2011), and with an increased risk for food allergies (Peterson, Frank, Pace, & Gordon, 2008; Sokol et al., 2008).

#### COMMENSAL BACTERIA ARE ACTIVE ENHANCERS OF GUT IMMUNE TOLERANCE

Food is a complex mix of many potential antigens; however, only a minimal amount (1% to 2%) is adsorbed through the mucosa in the antigens' intact immunogenic form. Therefore, maintaining tolerance against those potential antigens is one of the major defense mechanisms for the prevention of inflammatory bowel diseases. There are at least three different ways to maintain tolerance in the gut: first, by active immune suppression of immune responses through regulatory T cells (Tregs); second, by evasion of immune recognition operated by microbiota; and finally, by production of soluble factors (anti-inflammatory cytokines, IgA, and antimicrobial peptides [AMP] such as defensins). Recent studies have demonstrated that commensal bacteria participate in all of these defense mechanisms (Sansone & Medzhitov, 2009).

Active suppression can be mediated by bacteria through stimulation of Tregs, which are the most important immune T cells aimed at suppressing immune responses to microbe-triggered intestinal inflammation. For instance, it has been reported that several species of bacteria such as *altered Schaedler flora* resulted in a de novo expansion of mucosal Tregs in the colon lamina propria (Geuking et al., 2011). It seems that the encounter with commensal microbiota results in an increased generation of Tregs in the intestine rather than an induction of proliferation of pathogenic effector T cells (such as Th1 cells) that could deliver inflammatory signals. Thus, these data suggest that microbiota colonization-induced Treg cell responses are an essential intrinsic mechanism to promote and maintain host-intestinal microbial T cell interactions (Geuking et al., 2011). In addition, Lathrop and colleagues were able to demonstrate that those colonic Tregs express T-cell receptors different from those employed by Tregs in other body sites, suggesting a post-thymic education of immune cells in the periphery driven by commensal microbiota (Lathrop et al., 2011).

The escape from immune recognition can occur through a failure in the expression of virulence factors such as pathogen-associated molecular pattern receptors (PAMPs), includ-

ing lipopolysaccharide and peptidoglycan. It is still not clear if the diversity on the expression level of PAMPs between commensal and pathogenic bacteria can be considered a crucial mechanism that the immune system uses to distinguish between the two bacterial categories. PAMPs trigger a wide variety of pathogen-recognition receptors (PRRs), either extracellular, such as Toll-like receptors (TLRs), or intracellular, such as nucleotide-binding oligomerization domain receptors (NODs), both expressed on intestinal epithelial and immune cells (Maloy & Powrie, 2011). Basal activation of PRRs that leads to stimulation of intracellular pathways is essential to preserve intestinal cell homeostatic processes. These stimulated responses can include, for example, intestinal epithelial cell proliferation caused by the increase of anti-apoptotic factors, induction of tissue repair mechanisms, and production of protective factors such as AMPs—defensins and RegIII, for example (Vaishnavi, Behrendt, Ismail, Eckmann, & Hooper, 2008; Asquith, Boulard, Powrie, & Maloy, 2010; Maloy & Powrie, 2011).

Bacteria sensing is thought to induce a constant and necessary minimal level of “physiological inflammation” to ensure the health of our intestines throughout our lives. Any defects in bacterial sensing can lead to bacteria penetrating into the lamina propria, triggering substantially larger inflammatory immune responses. This hypothesis was widely demonstrated by different studies that used mice deficient in some TLRs, such as TLR4<sup>-/-</sup> mice (Rakoff-Nahoum, Paglino, Eslami-Varzaneh, Edberg, & Medzhitov, 2004) and TLR5<sup>-/-</sup> mice (Vijay-Kumar et al., 2007), or mice that do not express protein involved in their downstream activation pathways, such as Myd88<sup>-/-</sup> mice (Rakoff-Nahoum et al., 2004). All these groups of mice have been reported to be highly sensitive to dextran sodium sulphate-mediated colitis (Rakoff-Nahoum et al., 2004), and about 30% of TLR5<sup>-/-</sup> mice spontaneously develop severe gut inflammation with prolapse development (Vijay-Kumar et al., 2007). In the same way, activation of NOD receptors leads to the activation of transcription factor NF- $\kappa$ B and mitogen-activated protein kinase (MAPK)-pathways. Those pathways are highly modulated during different immune responses, including oxidative stress, inflammation, and bacterial or viral infections (Sansonetti & Medzhitov, 2009). The importance of these activation signalings in the protection against intestinal inflammation has been demonstrated in Crohn’s disease patients with mutations in the NOD2 gene that result in an impaired activation of NF- $\kappa$ B (Strober, Murray, Kitane, & Watanabe, 2006). Moreover, mice that do not express NLRP13, a protein belonging to the NOD family of receptors, also succumb to acute models of colitis (Zaki et al., 2010). All these findings indicate that the activation of PRRs by commensal bacteria is a necessary signal to coordinate intestinal immune responses. However, it is unknown how PRRs are able to differentiate between commensal and pathogenic bacteria and deliver different signals to the gut immune cells.

Finally, production of soluble factors, including anti-inflammatory cytokines such as IL-10 and TGF- $\beta$  and AMP mol-

ecules such as defensins, as potent tolerance inducers in the gut has been evaluated (Sansonetti & Medzhitov, 2009; Maloy & Powrie, 2011). Defects in the production of antimicrobial peptides increase the bacterial invasion, leading to subsequent inflammation. In addition, a specific subset of dendritic cells that express CD103 can stimulate Tregs to produce anti-inflammatory factors such as TGF- $\beta$  or retinoic-acid (Coombes & Powrie, 2008). While in mice, IL-10 deficiency results in a spontaneous development of colitis (Berg et al., 1996), in human beings, mutations in IL-10 receptor genes *IL10RA* and *IL10RB* have been recently found to aggravate IBD (Glocker et al., 2009). Likewise, exposition of *Bacteroides fragilis* protects us against the colitis induced by *Helicobacter hepaticus* through the stimulation of the anti-inflammatory cytokine IL-10 by intestinal immune cells (Mazmanian et al., 2008). *Clostridium* species also have been shown to actively promote IL-10 production (Atarashi et al., 2011).

Taken together, these findings indicate that gut homeostasis depends on maintaining the balance among all three major defense mechanisms that the gut uses: active immune suppression of immune responses by Tregs, microbiota immune recognition-evasion, and the production of soluble factors. Commensal bacteria are shown to be an essential component for gut health through all these mechanisms.

#### THERAPEUTIC EXPLOITATION OF COMMENSAL MICROBIOTA

If commensal bacteria are indeed important for the regulation of immune responses, they represent a novel area for therapeutic intervention in microbial imbalance (dysbiosis) conditions. Probiotics are “living microorganisms that, upon ingestion in sufficient numbers, exert health benefits” (Schrezenmeir & de Vrese, 2001). They can be included in dietary products such as yogurt. After oral administration, they are expected to survive the low pH in the stomach and to colonize the mucosal surfaces of the colon, albeit for a short period.

The most-common probiotic bacteria belong to the *Lactobacillus* and *Bifidobacterium* species, and they have been shown not only to improve and regulate immune-system responses but also to have a positive influence on the preexisting microflora stability, to inhibit pathogen colonization, and to enhance mucosal trophic mechanisms by stimulating intestinal epithelial cell barrier responses. As probiotics express PAMPs, they are thought to mimic the function of commensal bacteria by engaging and activating the PRRs on the epithelial mucosal surfaces. Confirmations of immunological probiotic functions were demonstrated by *Lactobacillus rhamnosus* and *Bifidobacterium lactis* having increased epithelial resistance, enhancing the phosphorylation of epithelial proteins such as occludin and ZO-1 (Mathias et al., 2010). Heat-shock proteins are constitutively expressed on epithelial cells and, under stress conditions, regulate the epithelial homeostasis (Petrof et al., 2004). It has been shown that the probiotic *Lactobacillus GG*

releases soluble factors that have a cytoprotective effect by stimulating synthesis of heat-shock proteins in intestinal epithelial cells through p38 and MAPK pathways (Tao et al., 2006). Many other natural anti-inflammatory properties have been described for probiotics, ranging from the capacity to increase lymphocyte proliferation to the stimulation of innate and adaptive immune responses, including IL-10 production. While some *Lactobacilli* strains are able to increase phagocytotic processes in macrophages (Maassen, 1999) and to stimulate the secretion of lysosomal enzymes (Perdigon, de Macias, Alvarez, Oliver, & de Ruiz Holgado, 1986), other strains have been found to regulate the production of pro-inflammatory cytokines, including IL-4 and IL-12 (Sütas, Hurme, & Isolauri, 1996). All these findings suggest that probiotics can stimulate both adaptive and innate immune responses. Ultimately, probiotics can be used not only to reinforce the natural mucosal barrier defenses but also to prevent an imbalance between pro-inflammatory and anti-inflammatory cytokines. Although the efficacy of probiotics in clinical trials for ulcerative colitis patients is promising, clinical trials in Crohn's disease patients have not inspired much enthusiasm (Shanahan, 2010).

In the last decade, an unconventional therapeutic approach has been used to treat a severe case of intestinal dysbiosis. This technique, called "fecal transplantation," aims to introduce microbes isolated from a donor gastrointestinal tract to the dysbiotic patient in order to establish more "normal" commensal flora. The pioneer of fecal transplantation is Dr. Lawrence Brandt (chief emeritus of gastroenterology at Albert Einstein College of Medicine), and so far his results have been quite encouraging (Palmer, 2011). Nevertheless, more studies need to be completed before we can fully understand the exact mechanisms that underlie the beneficial host-microbe interactions that seem to be established in those therapeutic approaches.

## CONCLUSION

Current studies regarding the interaction between commensal bacteria and gut immune and nonimmune cells suggest that gut microbiota are not ignorant bystanders in our intestines; they are one of the major contributors to the maintenance of intestinal homeostasis. Through these essential and constant host-microbial interactions, the overall digestive functions are maintained. Microbiota protect against infection because they can compete with opportunistic and pathogenic bacteria, but they also break down and digest food and stimulate cell-defense mechanisms. It is extremely important to maintain the balance among bacterial species. This bacterial biodiversity depends on the characteristics of the bacteria themselves and on the environment, including nutrition, tissue repair, and physiological processes. While low commensal bacteria biodiversity facilitates the entrance of pathogens, high microbiota diversity provides optimal conditions for a healthy digestive system.

There is still much to learn about how commensal bacteria regulate intestinal homeostasis. Understanding the regulation of mucosal immune responses to gut microbiota may be the key to targeted manipulation of immune cell-mediated responses, of nonimmune cell-mediated processes, and of microflora composition. Genetic approaches that could modify bacterial flora to selectively enhance the production of anti-inflammatory cytokines (e.g., IL-10) or antimicrobial peptides (e.g., defensins, RegIIIg) have already been considered as therapy for IBD patients (Steidler et al., 2003). However, improvements and combined studies on all the components of the digestive system, on their distinct functions, and on their cross-talks could create a successful strategy to treat intestinal inflammatory diseases.

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## Conflict of Interest Disclosure

The author has completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. No conflicts were noted.

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