Dear editor,

Please find enclosed the edited manuscript in Word format (file name: 4546-review.doc).

Title: "The intestinal barrier: a gentlemen's agreement between microbiota and immunity"

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Name of Journal: World Journal of Gastrointestinal Pathophysiology

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The manuscript has been improved according to the suggestions of the reviewers:

Reviewer #1

The review entitled "Intestinal barrier: a gentlemen's agreement between microbiota and immunity" gives a comprehensive overview about the current understanding. However, the section about the role on the microbiota on the intestinal permeability should be added. Moreover, the majority of publications lack the year.

Thank you for the suggestions. We have corrected our references.

We have also added a section on intestinal permeability on page 15, entitled "Gut permeability: an unclear connection between altered gut microbiota and immune system".

Reviewer #2

This is a very nice review of interactions between the intestinal microbiota and mucosal immune system. I do not have any major comments or concerns, but I do think that there are a few points that could be addressed. The last sentence in the first paragraph on Page 3 was not clear.

We agree with the reviewer. We have rewritten this sentence, as follows:

"This quantity makes explicit the clear mutual benefit for both the microbiota and the host".

Page 4, first full paragraph please delete de in de development.

We have deleted the word "de" on page 4.

IN the following sentence, Clostridium should be in italics.
We have formatted the word Clostridium in italics.

The section titled "How to recognize from friends" was not clear.
We agree with the reviewer. The title of this section has been changed to “Distinguishing enemies and friends: a visceral challenge”.

How do cells of the intestinal immune system discriminate between commensal and pathogenic microorganisms? Please comment whether cells of the mucosal immune system are not reactive towards commensal microbes, or whether they are largely ignorant of commensal microbes (due to the protective epithelial barrier).
We have added the following paragraphs in order to show the current knowledge about how the intestinal immune system can distinguish pathogens and commensals, as follows:

“Interestingly, the intestinal immune system is able to distinguish commensals from pathogenic microorganisms. Hosts can sense commensals differently than pathogens even though they have the same immunostimulatory molecules as pathogenic bacteria and are capable of triggering inflammation if they penetrate the intestinal epithelial barrier. Many studies have shown that this sensing of commensals is important for the development and functionality of the immune system because germ-free mice have reduced cellularity and impaired functionality of the immune system in the lamina propria of the small intestine [23].

Under normal conditions, the immune system is instructed by commensal microbiota to not respond to luminal antigens. Furthermore, commensal microbiota secrete metabolites by nutrient processing, prevent infections by pathogenic microbes, provide signals to induce healthy immune development, and stimulate innate and adaptive immune responses to maintain homeostasis. However, when dysbiosis occurs, non-invasive bacteria are transported to key immune inductive sites, the mesenteric lymph nodes (MLN) [24-30]. This abnormal situation leads to aberrant immune responses against microorganisms that otherwise would not be considered a threat.

The most important difference that distinguishes pathogens from commensals is the outcome of their interaction with the host. In the intestine, an infectious process usually starts with adhesion to the brush border of intestinal cells[31, 32]. After the adhesion phase, pathogenic bacteria produce virulence factors that are secreted in the external environment or injected into the cytosol of host cells. Non-invasive bacterial pathogens are able to inject virulence factors that contribute to the remodeling of the cytoskeleton of the host, leading to the formation of pedestal structures, which facilitate enhanced adhesion. Other pathogens

include invasive and facultative intracellular bacteria, which secrete virulence factors that enable these pathogens to cross the epithelial barrier[33] by remodeling the actin cytoskeleton. Thus, these bacteria are able to penetrate into host cells and form a specialized niche that increases their survival[34]. Importantly, invasive pathogens need to resist innate immune defenses, survive phagocytosis and, in some cases, manipulate adaptive immunity to cross the epithelial barrier and establish infection.

Certain components of the microbiota have been shown to lead to inflammatory responses, whereas others lead to anti-inflammatory mechanisms. The diversity and the composition of the microbiota thus play key roles in the maintenance of intestinal homeostasis and partially explain the link between intestinal microbiota changes and gut-related disorders in humans[3, 12, 13, 16, 35-37].

Indeed, an association has been established between changes in the relative abundance of certain bacterial groups and the unexpected responses of the human immune system leading to diseases. The opposite situation is also observed, in which introducing a bacterial type restores homeostasis[38]. For example, Faecalibacterium prausnitzii, a member of the normal human microbiota, has been associated with the extension of the period of remission in patients with Crohn's disease[39].

Gram-positive bacteria have microbe-associated molecular patterns (MAMPs), such as cell wall polysaccharides, peptidoglycans, lipoprotein anchors, lipoteichoic acids (LTA) and wall bound teichoic acids (WTA), that are capable of influencing pattern recognition receptor (PRR) recognition of known MAMPs, leading, for instance, to a shield effect[40, 41]. These MAMPs interact with PRRs, such as the Toll-like Receptors (TLRs), C-type lectin receptors (CLRs) and nucleotide oligomerization domain (NOD)-like receptors (NLRs), driving the induction of innate immune responses, with immune activation, antigen presentation, and expression of antimicrobial factors[42, 43].

Commensal bacterial components are usually recognized by TLRs, which is important for protection against gut injury and associated mortality. Impairment in the interaction between commensal bacteria and TLRs have been reported to promote chronic inflammation and tissue damage, e.g., inflammatory bowel disease[44]. There are two possible mechanisms by which TLR activation mediates this interaction: 1.) steady-state induction of protective factors via constitutive detection of lumen-derived microbial products by TLR2 expressed on colonic epithelium or 2.) upon epithelial damage, commensal-derived TLR ligands induce the production of protective factors. Recent studies have shown a role for CpG DNA, which is an agonist of TLR9, in mediating the beneficial effects of probiotics in the gastrointestinal tract[28]".

On page 5, it is stated that the epithelial barrier should not be damaged because of inflammatory responses triggered by the luminal contents. What is meant by this statement? Are the authors arguing that inflammation will not affect the epithelial barrier? There are many examples of inflammation disrupting tight junctional protein expression (for example), so this statement needs to be clarified.
Indeed, the sentence mentioned by the reviewer was written in a confuse manner. Therefore, we decided to delete it and improve the paragraph as follows:

"Under normal conditions, the immune system is instructed by commensal microbiota to not respond to luminal antigens. Furthermore, commensal microbiota secrete metabolites by nutrient processing, prevent infections by pathogenic microbes, provide signals to induce healthy immune development, and stimulate innate and adaptive immune responses to maintain homeostasis. However, when dysbiosis occurs, non-invasive bacteria are transported to key immune inductive sites, the mesenteric lymph nodes (MLN) [24-30]. This abnormal situation leads to aberrant immune responses against microorganisms that otherwise would not be considered a threat."

The section pertaining to lamina propria dendritic cells and macrophages was well written, but there was little mention as to how intestinal microbes might influence these populations. What is known about the role of the microbiota in shaping the development of lamina propria macrophages and dendritic cells?
The role of the gut microbiota in shaping the development of lamina propria and dendritic cells is still unclear and many studies are being conducted now to elucidate this question. We exposed this lack of information in some sentences of the section. Moreover, we changed the title of the section to "Intestinal Dendritic Cells and Macrophages: a complex distinction" in order to emphasize the aim of the section, that is showing how macrophages and dendritic cells may be distinguished in the intestinal lamina propria, according to recent studies.
The following paragraphs expose the lack of information in regards with the role of the microbiota in shaping macrophages and dendritic cells:

"Interestingly, Diehl et al.[83] showed that the CX_3CR1^{hi} mononuclear phagocytes of the intestine, which had previously been shown to be non-migratory, were able to migrate into MLNs in the absence of MyD88 or under conditions of antibiotic-induced dysbiosis in a CCR7-dependent manner, carrying non-invasive bacteria captured from the intestinal lumen and inducing both T lymphocyte responses and IgA production to avoid inflammatory bowel disease. The microbiota seem to instruct the immune system to inhibit migration of bacteria to MLNs via $CX3CR1^{hi}$ cells. This mechanism leads to tolerance to commensal bacteria."

"DCs constantly survey the microenvironment and coordinate a balance of maintaining immune tolerance to harmless antigens while mounting immune responses against enteric pathogens. Depending on from which bacterial strain components were derived, DCs can be stimulated, leading to either IL-12 secretion and a Th1 response, or IL-10 secretion and a Th2 response, as will be detailed below."

"The effects of gut microbiota in the cells of the lamina propria, which are crucial in recognizing bacterial tolerance induction and orientation of T cell responses, appear to be essential for the maintenance of intestinal immune homeostasis. The plasticity of dendritic cells, for example, is extremely important for their ability to respond to microbial stimuli and the ability to capture luminal bacteria and migrate to MLN. In the lamina propria, macrophages are educated to acquire non-inflammatory characteristics. Interestingly, however, the expression of $CX3CR1^{+}$ in macrophages that were isolated from colon differs considerably from those isolated from the duodenum, jejunum and ileum, suggesting that the instructions that macrophages receive from these regions are variable. This makes it clear that distinct commensal populations in different regions of the intestine give signals to these cells, influencing their profiles.[86]

The role of gut microbiota in macrophage and DC development is not clear. It is known that these cells participate in the regulation of intestinal immune responses against various microorganisms and diseases by producing several pro- and anti-inflammatory cytokines in an attempt to maintain intestinal homeostasis. This is an important topic for further investigation."

The use of REGIIIg was not consistent--please either use the gamma sign or g, not both.
Thank you. We have corrected the manuscript, using the gamma sign.

Reviewer #3

The manuscript 4546 entitled "Intestinal barrier: a gentleman's agreement between microbiota and immunity" submitted to World Journal of Gastrointestinal Pathophysiology attempts to review the literature concerning the role of the intestinal microbiota in modulating intestinal and sytemic health. The research area is immensely complex and knowledge gaps are abundant, making a review of the literature a highly ambitious task. Unfortunately, this review is more reminiscent of a very rough first draft rather than a polished, insightful review. It is very superficial, is lacking in organization and language precision, and is therefore exceedingly difficult to read. Apparently the authors mostly summarize findings, often far too briefly and superficially, without attempting to interpret their significance or how the findings may relate to each other. And these summaries appear

to have been rather simply and often haphazardly grouped together under often misleadingly titled sections. Thus the manuscript does not flow well. Often the section titles do not accurately describe the content of the section or the content does not live up to the section title. For example “How to recognize enemies from friends” on page 4: shouldn’t the role of MAMPs been placed here?

Thank you for you considerations. We have now improved our manuscript, adding more information and new interpretations in order to make our review suitable for this journal. We have also moved some paragraphs to other sections, such as the role of MAMPs, which became part of the section “Distinguishing enemies and friends: a visceral challenge”. Our manuscript has also been submitted to professional English editing.

In the sections that relate to intestinal immunity, I also often questioned whether the data cited was correctly interpreted by the authors, which leads to the impression that the authors’ main areas of expertise may not be within intestinal /mucosal immunology. This was confirmed by looking up the authors’ previously published articles, which indicate that they have their primary research interests in renal disease and obesity/metabolic syndrome. Perhaps the authors should consider including an author with more direct knowledge of the intestinal immune system?

The reviewer is right that previously published articles from our group belong to renal disease and metabolic syndrome. However, in the last two years, our group has been dedicated to studying intestinal immunology. Since we received an invitation from World Journal of Gastrointestinal Pathophysiology to write this review, we accepted this task as a possibility to deepen our comprehension on this subject.

Acronyms are often not defined. The references often lack the year they were published.

We have now corrected the definition of acronyms and the references.

Thank you again for publishing our manuscript in the World Journal of Gastrointestinal Pathophysiology.

Sincerely yours,

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