Format for ANSWERING REVIEWERS

April 07th, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9275-review.doc).

Title: *Escherichia coli* in chronic inflammatory bowel diseases: an update on Adherent Invasive *E. coli* (AIEC) pathogenicity.

Authors: Martinez-Medina, M and Garcia-Gil, LJ

Name of Journal: *World Journal of Gastrointestinal Pathophysiology*

ESPS Manuscript NO: 9275

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

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers:

Reviewer 1.

The authors welcome the suggestion of the reviewer and, in consequence, we have included a figure (Figure 1) about the putative impact of *E. coli* on IBD.

Reviewer 2.

We are grateful to the reviewer for her/his guidance in the revision of the manuscript. We have replied point by point to the issues and revised our manuscript accordingly. All changes are in red in the revised version.

Comment 1. We agree with the reviewer and thus we have changed the title as: "*Escherichia coli* in chronic inflammatory bowel diseases: an update on Adherent Invasive *E. coli* (AIEC) pathogenicity."

Comment 2. We thank the suggestion of the reviewer; we have included two new paragraphs with information extracted from three new references. We have focused on those studies related with AIEC, since a general discussion about dendritic cells and TLRs in IBD pathophysiology is a wide topic that we did not intend to discuss here. We hope the information included meets the requirements of the reviewer.

One paragraph summarizes a recent and very interesting work about the role of the intermediate filament vimentin in bacterial pathogenesis, which acts as a host cell receptor for AIEC (Stevens *et al.* Gut 2013). Vimentin would also interact with NOD2 in the intracellular side allowing NOD2 to localize near to the plasma membrane. In turn, NOD2 recruits the autophagy related protein ATG16L1. Interestingly, there is a link between NOD2 polymorphisms and the ability to interact with vimentin, being those NOD2 mutated proteins less able to interact with vimentin, thus remaining principally in the cytosol. The authors suggest that patients with mutations in *nod2* gene would be less able to mount a proper inflammatory response and to induce autophagy.

The other paragraph refers to AIEC behavior on monocytes isolated from Crohn's disease patients with different *nod2* and *tlr4* genotypes (Peeters *et al.* IJI 2007). The authors found an anomalous inflammatory response in CD patients with polymorphisms in *nod2* gene, but not in patients with a mutation in *tlr4*. Similar defective innate responses were observed in monocyte-derived dendritic cells from CD patients exposed to lipopolysaccharide (Nieminen *et al.* CEI 2014).

Comment 3. There is substantial consensus about increased *E. coli* in ileal CD but not in other IBD subtypes including UC. The increase in *E. coli* observed in some IBD patients frequently correlates with disease activity status and, as we and others have demonstrated, increased *E. coli* is related to reduced time until relapse in CD. Moreover, we have shown that anti-TNFα treatment leads to reduced *E. coli* populations whereas the abundance of other intestinal species such as *Faecalibacterium prausnitzii* is not restored (Lopez-Siles *et al*. IJMM 2014), being TNFα crucial in AIEC pathogenesis. Altogether, these works suggest that *E. coli* overcolonisation is associated with intestinal inflammation and the fact that *E. coli* imbalances are not a feature of all IBD subtypes suggests that *E. coli* is not just a cause of inflammation. Further studies are needed to determine whether *E. coli* plays an important role in colonic CD and UC. These ideas have been already presented in the review manuscript.

On the other hand, we are aware that the imbalance in *E. coli* can be a consequence of a primary inflammatory response. Actually, our hypothesis is that environmental factors such as diet are the cause of intestinal dysbiosis and a low-grade inflammation (Martinez-

Medina *et al.* Gut 2013). In this work we also showed that dysbiosis and a low-grade inflammation in susceptible individuals leads to increased colonization of adherent-invasive *E. coli*, what in turn exacerbates the inflammatory response and epithelial barrier disruption. Additional studies show that there is a need of microbial dysbiosis or intestinal inflammation for a proper AIEC colonization. This is evidenced because mice must be pre-treated with antibiotics (Carvalho *et al.* JEM 2009, Drouet *et al.* JTM 2011), DSS (Carvalho *et al.* IBD 2009) or western diet (Martinez-Medina *et al.* Gut 2013) to succeed with AIEC infection. The work of Craven *et al.* (PLOS One 2012), nicely shows that moderate to severe ileitis produced by protozoan infection in mice models induces dysbiosis and proliferation of endogenous mucosally invasive *E. coli*. However, a study on mice lacking the flagellin receptor TLR5 (T5KO mice, prone to developing spontaneous colitis) demonstrates that AIEC infection itself induces microbial dysbiosis that persists in the host and further results in chronic inflammation due to the presence of elevated levels of lipopolysaccharide and flagellin of the dysbiotic community (Chassaing *et al.* Gut 2013). Therefore, inflammation can instigate imbalances in this species, especially the AIEC pathotype, and these bacteria can in turn be involved in a further dysbiosis and increased intestinal inflammation. We have included a new section called "AIEC, a cause and a consequence of inflammation" to clarify this issue.

Comment 4. The reference has been included in the new version of the manuscript, as well as other references that were lacking.

Comment 5. We thank the suggestion of the reviewer. We have included a table (Table 1) and, accordingly, we have modified the text to clarify this issue. Actually, since the majority of works have included both CD and UC patients we think this disagreement is not a result of the methodological approaches used. Rather, these controversial observations could be attributable to differences in the disease severity of patients included in the studies because a number of studies that associate *E. coli* with UC specify that patients are in active phase when the dysbiosis is more evident. So, further works should consider each IBD phenotype and active/inactive patients as different groups for comparison.

Comment 6. As the reviewer states, the IFNγ/Jak/STAT1 pathway is an important mechanism of the host to respond towards most of the intestinal microbial species. Once bacteria enter the host, one of the first cells of the immune system it encounters are macrophages, and IFNγ is one of the most important stimuli required for macrophages to mount an appropriate microbiocidal response. Moreover, an imbalance in the activity of STAT1 leading to STAT1 suppression or overexpression can disrupt host immune function, what predispose individuals to chronic infections.

However, not all intestinal bacteria are able to subvert the IFNγ signal transduction pathway. The authors studied the capacity of EHEC CL-56 O157:H7, EPEC E2348/69

O127:H6, three AIEC strains (LF82, O83 and UM146), and the commensal *E. coli* strain HB101 to avoid phosphorylation of the activator of transcription STAT-1, but only the EHEC and the AIEC strains had the ability to block STAT-1 phosphorylation. That means this mechanism is not common in intestinal bacteria such as commensal *E. coli*. Moreover, the authors showed in a previous study that shiga toxin is in part responsible of this mechanism of pathogenicity. However, this virulence factor is not present in AIEC, what suggests that AIEC may act differently. The authors searched for a secreted molecule because AIEC internalization was not required for the bacteria to subvert the IFNγ signal transduction pathway, and they concluded that a small secreted peptide is the responsible because a molecule heat-resistant and proteinase K sensible present in culture supernatants mediated inactivation of STAT1 signaling. The paragraph has been extended to include this information.

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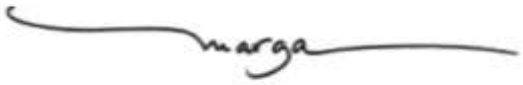
3 References and typesetting were corrected

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

Sincerely yours,



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