Author's response to reviews

Title: Transcript levels of Toll-like receptors 5, 8 and 9 correlate with inflammatory activity in Ulcerative Colitis

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Author's response to reviews: see over
Dear Editor:

Enclosed you will find the revised version of the manuscript entitled "Transcript levels of Toll-like receptors 5, 8 and 9 correlate with inflammatory activity in Ulcerative Colitis" and the response for the reviewers, which comments were very helpful for the manuscript. All reviewer’s suggestions were incorporated in the text.

I am looking forward hearing from you soon.

Best regards,

Prof. Jesus K. Yamamoto-Furusho M.D., Ph.D.

Head of IBD Clinic

Department of Gastroenterology
Response to Reviewers

Response to reviewer Mathias Chamailard:

Is there any abnormal responsiveness to TLR5, TLR8 and TLR9 agonists of colonocytes from UC patients with quiescent disease?

We considered your research question to be very insightful; however we found that only TLR4 shows a significant difference between healthy controls and quiescent UC patients. Other TLRs only showed trend. For the TLR8 case, Steenholdt does not mention about agonist treatment in colonic epithelial isolated cells. To our knowledge in the TLR5 and TLR9 case, this has not been explored yet.

Is there any influence of the therapeutical management of the UC patients on changes of TLR5, TLR8 and TLR9 transcript levels?

We evaluate TLRs mRNA levels according to treatment and found that TLR5 mRNA levels were lower in the patients with severe activity assessed by both endoscopic and histological, and now discuss it as follows: we found 30% less TLR5 mRNA levels in the endoscopic (moderate and severe) and histological (moderate and severe) activity treated with corticosteroids compared to the patients treated only with 5-ASA (p=0.04), in the first paragraph on page 10, from the discussion.

In the abstract, the authors wrote that 50 UC patients were included in their study while in the methods section they mentioned that rectum biopsies were collected from 60 UC patients.

We corrected this part in the manuscript and the correct patients number of patients studied was 51.
Response to reviewer Ingrid Arijs

The numbers of the studied patients are different in the text and the table.

**We corrected the number from 50 to 51.**

Please put all the p-values between brackets.

**P-values are now presented in the text between brackets (P=…)**

Please write the references according to the instructions of the journal.

**References are now presented according to the instructions of the journal recommendations, using EndNote.**

The mentioned authors in the text are not correctly written or are not the same as in the references (e.g. Steenholdt, Stanislawowski,)

**This is now corrected in the text.**

Written errors: please check the whole text, e.g. Spearman instead of Sperman. assess instead of assess’, TOLLIP instead of Toll on page 6, chemokines instead of chemokine’s

**This has been corrected on text.**

Check the comma’s and points (the authors forgot a point between Smirnoff and Differences under the paragraph ‘Analysis’ in the methods section) in the text, and the construction of some sentences (some are too long and some are not correct, e.g. first sentence on page 4: ‘demonstrated TO BE important’)

**This has been corrected.**

Human gene symbols need to be written in italic

**The Human gene symbols are now in italic throughout the document.**

The text of the methods is not clearly written, especially the qPCR text and analysis text.

**In order to clarify this, we wrote another version for the RT-qPCR.**

The figures are not clear: please add what the x-axis and the y-axis are showing

**We change the descriptor in the figures 1-3 and 4 and 5 for x-axis: to be healthy endoscopic and/or histological activity and for y-axis: TLRs relative expression, according to 2-ΔΔCt..**

Please give the reason of the endoscopy of the controls.
Controls inclusion criteria and the reason for the endoscopy is now explained in the methods section.

Please add medication and disease activity (riley scores) in table 1.

This has been added in the text and in table 1.

In table 1, the values of age are median and interquartile range?

The age values are now specified in table 1.

In the introduction test, the sentence ‘Most studies in IBD have focused only in TLR2, TLR3 and TLR4, but other TLRs expression has not been extensively explored in the colonic mucosa form UC patients’ is mentioned twice.

This error has been deleted.

Disease is missing in Crohn’s disease on page 3 in second a linea.

This has been corrected.

In the discussion, the reference of Cario is missing in the text.

This has been corrected.

Besides the figures, please make a table of the qPCR results showing the p-values of the comparisons of TLRs between the different groups.

We now submit a manuscript version that includes these results in table 3.

Furthermore, it would be nice to confirm the results on protein level and in an independent cohort.

We are considering doing so for a different paper, although the previous studies in TLRs have shown good correlation between mRNA levels and protein levels.