Reviewer’s report

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

Version: 4 Date: 9 March 2011

Reviewer: Ingrid Arijs

Reviewer’s report:

Comments for the revised manuscript:
- Abstract:
  - Results section (page 2):
    # TLR8,9 and IL6 upregulated in UC versus controls: overall UC or active UC?
    # IL6 qPCR results not mention in the results section of the manuscript.
    # TLR5,8 and 9 lower in quiescent than in active UC (p<0.05):
      • The mentioned p-value (<0.05) is not correct for TLR5, it is p-value=0.06 which is not significant
    # What about the other TLR’s, you mention only a few:
      • TLR2,4 is also upregulated in active UC vs controls and quiescent
      • TLR2,4 is also lower in quiescent than in active UC
- Methods:
  - Population and tissue samples (page 5):
    # Please mention table 1 in this paragraph
  - Sample processing RNA extraction and cDNA synthesis (page 6):
    # Please add ‘and’ before ‘by spectrophotometer
    # The complete affiliation of Thermo Scientific
  - Real-time RT-qPCR (page 6):
    # Please add ‘from’ before ‘Invitrogen
    # Please add ‘were used’ or another verb before ‘for relative quantification’
    # Please add ‘was performed’ or another verb before ‘using the Lightcycler’
    # Please clarify ‘calibrator’ in a separate sentence
  - Statistical analysis (page 7):
    # Please add a point behind ‘program’ and start a new sentence with ‘Statistical significance..’
    # Add a comma behind ‘differences’
    # Replace ‘and’ by a comma between TLR1 to 9 and TNF
- Results:
  o TLR1 to 9 mRNA profiles in UC compared with controls (page 8):
    # Please add the qPCR results of TNF and IL6 also in Table 3
    # Figures 1,2 and 3 are totally not clear:
      • Please add IN the figure not in the legend: the x-axis, the y-axis, the bars representing which genes?, the bars are the median and the interquartile range???
      • Please add in the figure what is significant
      • Please add in the legend what the bars representing: the median of the expression and the interquartile range???
      • Please add in the legend a point behind ‘controls’ and start a new sentence with ‘Differences..’.
      • Please add a comma before ‘and statistical significance’
    # Table 3:
      • Please add in the legend what you are meaning with underlined numbers and numbers with a star
      • Please add ‘UC’ behind ‘quiescent’ in the last column of the table
      • The transcript levels are given in means and standard deviations or median and interquartile range. Mention this in the legend of the table
    # Please make one sentence from the second and third sentence e.g. ‘We found a significant increased expression of TLR2, TLR4, TLR8 and TLR9 mRNA levels in patients with active UC when compared to patients with quiescent UC and healthy controls.
    o Moreover the first mentioned p-value in the original sentence is not correct, it needs to be p-value <0.05 instead of p<0.02) because it is p-value 0.04 between quiescent and active UC.
    # Please rewrite the sentence ‘TLR3 mRNA levels…’ to for example TLR3 mRNA showed no change in expression between the different groups.
    # Please rewrite the next sentence as followed:
      o E.g. ‘Comparisons between the UC quiescent group and the control group only showed a significant up-regulation for TLR4 mRNA levels, while the TLR2, TLR8 and TLR9 mRNA levels were similar (p=0.014)
      o The mentioned p-value is not correct in the orginal sentence, it is p=0.015 instead of p=0.04 (based on results in table 3)
      # I would delete the following sentence ‘Although the TLR2, TLR8 and TLR9 mRNA levels were similar ….’ Because you mention in the sentence before that there is ONLY one significant between controls and UC quiescent.
      # Mention that the lower expression of TLR5 in quiescent UC vs active UC is borderline significant (p=0.06)
      • You did not mention that TLR8 and TLR9 were significantly downregulated in
quiescent UC vs active UC.

# Please delete the comma before 'and' between TLR4 and TLR8 in the penultimate sentence of this paragraph.

• Add in the table 3 the qPCR results of comparison between the overall UC group versus controls

# Can you mention between brackets which other TLR's showed no statistical significance.

o Correlation of TLR5, TLR8, TLR9 and IL6 mRNA levels with endoscopic and histological activity (page 8-9):

# Can you give the reason that you only look for the correlation for TLR5, TLR8, TLR9 and IL6, and not for the other TLRs and TNF?

# Figure 4 and figure 5:

• The same comments that I made for the figures 1-3 (see above)

# The last sentence is not clear. What are you meaning? Can you give it in a figure? There is a lower TLR expression in patients with steroid treatment?

o TLRs mRNA levels correlate with IL6 and TNF mRNA levels in the colonic mucosa from UC patients

# IL6 in second sentence is not written in italic

# You mention a significant correlation between TLRs mRNA levels and IL6 and TNF, in particular with TLR5, TLR8 and TLR9. Not only TLR5, 8 and 9 but also TLR1, 2, 4 and 6. Why don't you mention these TLRs?

- Discussion (page 10-12):

o First sentence:

# The authors mentioned that TLR5 is up-regulated in active UC but this is not seen in table 3, only borderline significant (p=0.06) in active UC vs quiescent UC.

# Up-regulated in active UC versus controls or versus quiescent UC or versus both controls and quiescent UC.

# Please add 'in' before 'active UC patients'

o Didn’t the authors forget something behind ‘stimulated’ in the ‘TLR8 induction in human isolated epithelial cells stimulated has been reported’

o The correlation between TLR8 and TLR9 gene expression is only mentioned in the discussion, not in the results. Please mention it also in the results section.

o There is not only a trend of down-regulation for TLR5 in UC quiescent vs controls, but TLR6 shows also a trend of down-regulation in quiescent UC versus controls (it is more significant for TLR6 than for TLR5). Moreover, the mentioned p-value is not correct for TLR5, it is p=0.014 instead of p=0.011 (based on table 3).

o Why do mention only

o The authors only discussed the findings of the previous studies for TLR5, TLR8 and TLR9 but not the other TLRs. E.g. The findings of TLR2 and 4 in this study
are these findings the same as in other published studies.

Please replace ‘In a paper published by Stanislawowski et al., found…’ with ‘Stanislawowski et al. found…’

The sentence ‘This finding might be due to a down regulation of TLR5 in patients receiving steroids…’ is not clear.

Moreover, these findings are not mentioned in the results section. Can you add this to the results?

Furthermore, I cannot find the results in the results section of the upregulation of TLR5 in patients with pouchitis after total proctocolectomy.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests