Reviewer's report

Title: Effects of Interleukin-4 or Interleukin-10 Gene Therapy on Trinitrobenzenesulfonic Acid-Induced Mouse Colitis

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Reviewer: Fermin Sanchez de Medina

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The manuscript by Xiong et al. describes a gene therapy experiment on the TNBS mouse model of colitis. Their results indicate that IL-4 or IL-10 increase is beneficial, while combined therapy is actually deleterious, despite a positive effect on TNF and IL-6. The study is interesting, generally well conducted and presented. I have some comments as follows:

Major comments

1. The design leaves off a normal group. This is not absolutely required, but it would give a reference on the degree of inflammation, i.e. what is the score of a noncolitic mouse? According to methods this could easily reach 1-2. Also relevant for cytokines.

2. Another aspect of the design is the fact that half a gene dose was used in the combined group. This complicates the interpretation of the results.

3. My own interpretation of the data differs from that of the authors. First, the only colonic effect of IL-4 or IL-10 transfer is possibly on IFN-g (statistical analysis was apparently not performed except against the TNBS group). Histological score was actually reduced by the vector alone, and it is unclear whether gene carrying plasmid had an additional effect or not. But it is indisputable that TNF and IL-6 are identical. Thus the main impact is on body weight/DAI. Taken together with the intraperitoneal pathway, these data suggest that the effect if systemic rather than strictly colonic.

4. Why didn’t the authors measure IL-10 and IL-4 by qPCR? This is incomprehensible (to say the least) since they used the technique for other not so relevant endpoints.

5. Transfection technology was used very effectively in this study. Although vaguely familiar with this approach, I was surprised to see this successful outcome. It would be interesting to complement the manuscript with any more data the authors have, or alternatively to provide some insight from previous papers.

6. IL-4 is expected to produce inflammation – it is an inflammatory cytokine, although it tends to limit Th1 responses and in such context it can be anti-inflammatory.

7. The model used may or may not be acute in nature. In mice, as far as I know, a single dose of TNBS/ethanol without previous sensitization produces a
response similar to that in the rat, peaking at 2-4 days with necrosis and fibrosis of the tissue, followed by slow recovery. After a few days, depending on the dose, the colon is scarred and physiologically altered but otherwise essentially normal. When sensitization and sometimes repeated doses are carried out, the outcome is more chronic, and at least partially this seems to be the case here. Thus the model should be better described in this regard (ref. Nat Protoc. 2007;2(3):541-6).

8. The discussion was nicely written in that it kept to the point and avoided overinterpretation. However it should be now amended to feature the comments above (or rebuttal).

Minor comments
1. The order of presentation in Fig. 1 is not adequate. As mentioned above, Fig. 1 should actually feature qPCR data.
2. The Background section may be shortened a little.
3. Please do not use ‘pathologic’ to refer to histological analysis. Although widespread, all the measurements provided are indeed ‘pathologic’, aren’t they?
4. Please revise the manuscript for some typos (minor) and weird expressions.
5. Please refer always to the combination group as IL-4+IL-10 or ‘combination’ group.
6. Is combined results and discussion allowed in the journal?

Level of interest: An article of importance in its field

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

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

No competing interest