Author's response to reviews

Title: Therapeutic effect of all-trans-retinoic acid (at-RA) on an autoimmune nephritis experimental model: role of the VLA-4 integrin

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

Enclosed please find our revised manuscript entitled “Therapeutic effect of all-trans-retinoic acid (at-RA) on an autoimmune nephritis experimental model: role of the VLA-4 integrin”, with number of reference 7806347241193448. The revised manuscript addresses most of the Minor Essential Revisions required by the second Reviewer Dr. David Power.

We detail, in the point-by-point reply, all the modifications included in the manuscript and the figures. In addition, we also provide comments to reviewer’s suggestions.

1. “The time of administration of the at-RA needs to be stated in the Methodology. Presumably, it was given from the start of the induction of the model.”

In the Material and Methods section, in page 8 last line, it is clearly stated now that at-RA is applied from the beginning of the experimental protocol: “The following two groups of rats (Groups II and III) were treated since day 0 of the experimental protocol with chow pellet supplemented with at-RA (15 mg/Kg body weight)”

2. “Statistics for the comparison of cellular infiltrates in the various groups (page 15) should be performed”.

We have performed the statistical analysis required. Under Results section, page 15, lines 14-15, it is expressed the significance from the comparison of cellular infiltrates between HgCl2-injected rats (group I) and HgCl2-injected rats also treated with at-RA (group II) as follows: “Statistical significance comparing CD3+ and CD68+ infiltrated cells quantification between group I and group II was found (p<0.001)”.

3. “For Figure 4, the statistical comparison should be between the rats with nephritis that are either treated (group I) or not treated (group II) with at-RA.”
Comparisons with vehicle treated rats are not really the point of the experiment, and simply give a baseline. The asterisk is also placed wrongly in Fig. 4B”

Statistical comparison between HgCl₂-injected rats (group I) and HgCl₂-injected rats also treated with at-RA (group II) has been performed and indicated by asterisks in figure 4 panel A and B. Statistical significances are also expressed in the legend of figure 4, page 33, lines 8 and 10.

5. “The legend to Figure 5 should state what the two lines are in the flow cytometry histograms”.

In page 33 line 13-15 is now indicated what is represented by the two lines of the flow cytometry histogram, as follows: “Flow cytometry analyses were performed using anti-α4 Ab (HP2/1) (continuous line) and anti-mouse IgG Ab as a negative control (discontinuous line)”.

6. “Statistical analysis should be applied to Figure 6”.

Statistical analysis between K562-α4 cells treated with different concentrations of at-RA and K562-α4 untreated cells have been performed and the significance is indicated by means of asterisks in figure 6. Statistical significances are also expressed in the legend of figure 6, page 34 last line.

7. “In the Discussion, the authors should mention that the treatment was started prior to induction of the model (or so I assume), so the most important factor in the reduced damage with at-RA was probably reduced initial injury. To propose a significant contribution for other protective effects (eg. reduced leukocyte migration)
the experiments would have to be repeated and at-RA given after the initial induction of the model had been performed”.

The experimental model of autoimmune nephritis induced by HgCl$_2$ in BN rats has been extensively used in our laboratory (Molina et al., J. Immunol 153: 2313-2320, 1994; Escudero et al., JASN 9 (10): 1881-1891, 1998; Nieto et al., JASN 13 (4): 937-45, 2002). Our experience indicates that is quite complex to discriminate when exactly both autoimmune and inflammatory responses are triggered during the development of the disease. Additionally, as we mentioned in the discussion, both responses are connected and share common elements. Moreover, there is also an early tubular damage associated to mercury toxicity which may also contribute to the development of the autoimmune and inflammatory responses later on. Based on this, it is very difficult to establish different starting points of at-RA treatment in order to distinguish the differential effect of this compound in each of these responses. Therefore, we considered the most appropriate the administration of at-RA from day 0 of the experimental protocol, to examine the effect of at-RA in the autoimmune nephritis development and outcome.

Based on the results presented in this work, we agree with the reviewer that for a possible therapeutic application in developing autoimmune nephritis, further studies including different administration timings of at-RA application would be necessary.

Regarding to the Discretionary Revisions suggested: “The data from groups III and IV are not very important in some of the Figures, and one or other of the two groups could be removed at the discretion of the authors. This is so in Figures 3B and 5B”.

The authors decided to maintain the results obtained for all the experimental groups in all the figures. We agree with the reviewer that the modifications found for some of the
parameters using group III (at-RA) and group IV (vehicle) are not significant but we consider they are necessary controls to rule out any non-specific effect due to the at-RA treatment by itself or to the technical maneuvers (vehicle).

To facilitate the revision, all the modifications and the new comments included in the manuscript are marked in yellow along the document attached entitled “MM. Escribese et al., Revised Manuscript”.

Finally, we are grateful for your consideration of our work for revision and we hope that the revised manuscript is now acceptable for publication in your Journal.

Sincerely yours

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