European Journal of Immunology

# Peer review correspondence

**A pro-inflammatory signalome is constitutively activated by C33Y mutant TNF receptor 1 in TNF Receptor Associated Periodic Syndrome (TRAPS)**

Ola H. Negm, Heiko A. Mannsperger, Elizabeth M. McDermott, Elizabeth Drewe, Richard J. Powell, Ian Todd, Lucy C. Fairclough and Patrick J. Tighe.

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Handling Executive Committee member: Prof. Iain McInnes

Please note that the correspondence below does not include the standard editorial instructions regarding preparation and submission of revised manuscripts, only the scientific revisions requested and addressed.

First Editorial Decision – 6 January 2014

Dear Dr. Todd,

My apologies for the delay in processing the review of your manuscript ID eji.201344328 entitled "Constitutive activation of a pro-inflammatory signalome by C33Y mutant TNF Receptor 1 in TNF Receptor Associated Periodic Syndrome (TRAPS)", which you submitted to the European Journal of Immunology, due to the holiday season. The paper has been reviewed and the comments of the referees are included at the bottom of this letter.

Although the referees have recommended publication, some revisions to your manuscript have been requested. Therefore, I invite you to respond to the comments of the referees and revise your manuscript accordingly.

You should also pay close attention to the editorial comments included below.

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If the revision of the paper is expected to take more than three months, please inform the editorial office. Revisions taking longer than six months may be assessed by new referees to ensure the relevance and timeliness of the data.

Once again, thank you for submitting your manuscript to European Journal of Immunology. We look forward to receiving your revision.

Yours sincerely,
Karen Chu

on behalf of Prof. Iain McInnes

Dr. Karen Chu
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Reviewer: 1

Comments to the Author
The authors of this manuscript have proposed a pro-inflammatory signalome to describe the constitutive up-regulation of a wide spectrum of signalling intermediates and their phosphorylated forms resulting from the effects of the C33Y TRAPS-associated mutant form of TNFR1 on inflammatory signalling pathways.

They have applied proteomic techniques to study intracellular signalling pathways affected by this particular mutation. The prototypic mutant TNFR1 (C33Y), and wild-type TNFR1 (WT), were expressed at near physiological levels in an SK-Hep-1 cell model. Reverse Phase Protein Microarrays (RPPA) were carefully optimised to reveal constitutive activation of multiple inflammatory signalling pathways in these cells; this was corroborated by western blotting.

Although this work was done on a single C33Y TRAPS-associated TNFR1 mutation and would need to be confirmed in a range of dissease-associated TNFR1 mutations this is the most comprehensive picture to date of the pro-inflammatory signalome that is constitutively activated by a mutant form of TNFR1. Furthermore these observation were corroborated in PBMC of C33Y-variant TRAPS patients where a modest constitutive up-regulation of a wide spectrum of signalling intermediates and their phosphorylated forms associated with a pro-inflammatory/anti-apoptotic phenotype was observed despite these C33Y

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TRAPS patients being symptom free and treated with glucocorticoids and/or cytokine-blocking biologics. In future it will be interesting to see to what extent this signalome is specific to TRAPS and how it may differ from other monogenic autoinflammatory conditions.

The authors are to be complimented on a nicely constructed and well written paper – unfortunately this is often much more honoured in the breach than in the observance.

Reviewer: 2

Comments to the Author
This study was performed to further investigate the signalling pathway perturbation in TRAPS. TNFR1-associated signalling pathway intermediates were examined by utilising a prototypic mutant TNFR1 (C33Y), and wild-type TNFR1 (WT) in an SK-Hep-1 cell model and in PBMCs from C33Y TRAPS patients and healthy controls. In C33Y-TNFR1-expressing SK-Hep-1
cells and TRAPS patients' PBMCs, the authors have found an up-regulation of a wide spectrum of signalling intermediates.
This is an excellent study, probably the first demostrating the pleiotropic effect of a TRAPS-associated mutant form of TNFR1 on multiple inflammatory signalling pathways – a pro-inflammatory signalome.
The study was well designed, with convincing objectives and appropriated methodology; the manuscript is well written. However, the only concern for this referee is the type of patients selection. The authors should clarify if PBMCs from untreated patients and/or with the presence of inflammation could have induced a different response. Probably, at least in the future agenda, the authors should include in study TRAPS patients before and after the treatment

First revision – authors' response – 9 February 2014

Reviewer 1
In response to reviewer 1, we have added the following statements to the Discussion:
"It will be interesting and important to extend these observations to cells expressing other TRAPS-associated TNFR1 mutations, as we are currently doing for the R92Q variant of TNFR1 (Abduljabbar et al., in preparation)."
"Delineation of signalomes in other autoinflammatory diseases will also help to clarify whether the signalome defined in this study is TRAPS-specific, or shares features with other autoinflammatory diseases."

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Reviewer 2

In response to reviewer 2, we have added the following statement to the Discussion:

“It would also be desirable to extend the current studies to TRAPS patients with different levels of diseases activity, and before and after treatment with particular therapeutic agents to see how this affects the signalling pathways studied.”

Second Editorial Decision – 11 March 2014

Dear Dr. Todd,

It is a pleasure to provisionally accept your manuscript entitled "A pro-inflammatory signalome is constitutively activated by C33Y mutant TNF receptor 1 in TNF Receptor Associated Periodic Syndrome (TRAPS)" for publication in the European Journal of Immunology. Please accept my sincere apologies for the prolonged delay in processing the re-review of your manuscript.

For final acceptance, please follow the instructions below and return the requested items as soon as possible as we cannot process your manuscript further until all items listed below are dealt with.

Please note that EJI articles are now published online a few days after final acceptance (see Accepted Articles: http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1521-4141/accepted). The files used for the Accepted Articles are the final files and information supplied by you in Manuscript Central. You should therefore check that all the information (including author names) is correct as changes will NOT be permitted until the proofs stage.

We look forward to hearing from you and thank you for submitting your manuscript to the European Journal of Immunology.

Yours sincerely,
Laura Soto Vazquez

on behalf of Prof. Iain McInnes

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