European Journal of Immunology

FKBP51 modulates steroid sensitivity and NFκB signalling: A novel anti-inflammatory drug target

Marc Kästle, Barbara Kistler, Thorsten Lamla, Tom Bretschneider, David Lamb, Paul Nicklin, David Wyatt

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Review Timeline:	Submission date:	17-May-2018
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	Revision/s received:	12-Jul-2018
	Accepted:	23-Aug-2018

Handling Executive Committee member: Prof. Francesco Annunziato

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 - 04-Jul-2018

Dear Dr. Kästle,

Manuscript ID eji.201847699 entitled "Modulation of steroid sensitivity and NFκB signalling by FKBP51: A novel anti-inflammatory drug target" which you submitted to the European Journal of Immunology has been reviewed. The comments of the referees are included at the bottom of this letter. We are sorry for the delay in the peer review, we have received one of the reports with a major delay.

You will also note that both referees had difficulties navigating through the manuscript and one of the referees could not provide a full report before these issues are rectified. A revised version of your manuscript that takes into account the comments of the referees will be reconsidered for publication. Should you disagree with any of the referees' concerns, you should address this in your point-by-point response and provide solid scientific reasons for why you will not make the requested changes.

You should also pay close attention to the editorial comments included below. **In particular, please edit your figure legends to follow Journal standards as outlined in the editorial comments. The number of

experiments and mice per experiment in Figure 1 was not stated. Please ensure that data in this figure are statistically robust, and if this was not the case, experiments need to repeated more than once to ensure reproducibility. Please also state the number of mice/samples/replicates per experiment in all figures and panels in which this was not indicated. Failure to do this will result in delays in the re-review process.**

Please note that submitting a revision of your manuscript does not guarantee eventual acceptance, and that your revision will be re-reviewed by the referees before a decision is rendered.

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 and we look forward to receiving your revision.

Yours sincerely,
Nadja Bakocevic

On behalf of
Prof. Francesco Annunziato

Dr. Nadja Bakocevic
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Reviewer: 1

Comments to the Author
I tried to understand the paper flow. Unfortunately there is no correspondence between figure and text. The figure numbering does not make any sense. I tried to catch the flow of the paper but it is almost impossible. The labelling of the figures is not complete, so it is very hard to figure out why the columns have different aspects in the figure I assume is Fig.1 (according to the figure legend). Moreover, Fig. 3 shows the same problem, I am not sure I can assume that the columns have the same legend as in fig.2

and, in brief, there are similar issues through all the other figures.

Reviewer: 2

Comments to the Author

For many years, glucocorticoids are known as anti-inflammatory compounds that are broadly used to dampen inflammation. However, in spite of numerous studies it is quite unclear how they act in detail, at the molecular level. While by binding to steroid receptors that act as transcription factors they are able to enhance gene transcription, by trans-repression through the binding of ligand-bound glucocorticoid receptors they are able to affect â€“ to inhibit - the transcriptional capacity of NF-kB and AP-1 factors. Un-ligated glucocorticoid receptors can be sequestered in cytoplasm by chaperons, such as by HSP90 and the FK506-binding proteins FKBP51 and 52 that modify the action of steroids. Surprisingly, both FKBPs seem to affect transcription factors in various â€“ or even opposite ways. The goal of the current study was to elucidate how FKBP51 affects steroid sensitivity and, in particular, NF-kB activation in a steroid-dependent manner. They authors show in immune precipitations using anti-FKBP51 antibodies followed by MS assays that in cytosol complexes exist that consist of FKBP51, glucocorticoid receptors and members of IKK (NF-kB kinase) family. Inhibition of FKBP51 expression by siRNAs resulted in a marked reduction in NF-kB activation and, therefore, in a reduced expression of NF-kB target genes. Therefore, FKBP51 is able to dampen markedly the effect glucocorticoids.

In my view, the results of immune precipitations are the strongest part of the study and improve our view on the cellular activity of glucocorticoid activity. Therefore, this manuscript would fit well in the scope of EJI but a few more formal weak points should be corrected before acceptance of the manuscript for press. Thus, please correct the order of figures in the text. It is quite unusual to start with Fig. 3 in the text and, then, to jump back to Figs. 1 and 2. Why you could not start with some words on the immunization scheme - that you show in Fig. 1A - and develop your story in a more logical way? Moreover, due to the convincing other data, in my view you can delete Fig. 6 about the „negative data“ of pharmacological inhibitors of FKBP51, iFit4 and SAFit2, which did not show any effect on NF-kB activation in your hands. Two further points should also be corrected: Please avoid on page 3 the term „for the first time“ - in each case a novel publication should show a novel finding „for the first time“, otherwise it’s not worth to be printed. And in Fig. 1 (or is it Fig. 3?) please correct the typing error of „neutrophils“.

European Journal of Immunology

First Revision – authors' response -

Reviewer: 1

Reviewer: "Comments to the Author
I tried to understand the paper flow. Unfortunately there is no correspondence between figure and text. The figure numbering does not make any sense. I tried to catch the flow of the paper but it is almost impossible. The labelling of the figures is not complete, so it is very hard to figure out why the columns have different aspects in the figure I assume is Fig.1 (according to the figure legend). Moreover, Fig. 3 shows the same problem, I am not sure I can assume that the columns have the same legend as in fig.2 and, in brief, there are similar issues through all the other figures."

Author: We apologize for the confusion we made by mixing up the figures and the corresponding figure-references in the text. We corrected this mistake and hope that the manuscript is easy to follow now.

Reviewer: 2

Reviewer: "In my view, the results of immune precipitations are the strongest part of the study and improve our view on the cellular activity of glucocorticoid activity. Therefore, this manuscript would fit well in the scope of EJI but a few more formal weak points should be corrected before acceptance of the manuscript for press. Thus, please correct the order of figures in the text. It is quite unusual to start with Fig. 3 in the text and, then, to jump back to Figs. 1 and 2."

Author: We apologize for the confusion we made by mixing up the figures and the corresponding figure-references in the text. We corrected this mistake and hope that the manuscript is easy to follow now.

Reviewer: "Why you could not start with some words on the immunization scheme - that you show in Fig. 1A - and develop your story in a more logical way?"

Author: We also appreciate the help to generate a more logical flow for our story. We explained the experimental scheme for the HDM-in vivo model more precisely in the introduction of Fig.1 A now.

Manuscript text: "…FKBP51-AAVs were instilled intra-tracheally to HDM sensitized mice on day 4 (see Fig. 1A). On the last two days before sacrificing the mice to get the bronchial alveolar lavage fluid, we challenged the mice daily with 25µg HDM intratracheally to trigger an inflammatory lung response. Animals of the steroid groups got prednisolone b.i.d. on the same days…"

Reviewer: "Moreover, due to the convincing other data, in my view you can delete Fig. 6 about the "negative data" of pharmacological inhibitors of FKBP51, iFit4 and SAFit2, which did not show any effect on NF-kB activation in your hands."

Author: We thank the reviewer for this very good comment. We agree that Figure 6 does not fit to the flow of the paper. We did not want to hide "negative data", nevertheless we also think this is more related to the missing efficacy/characterization of the tested tool compounds and not the concept per se. Thus, we follow the suggestion and deleted Figure 6 and the corresponding parts in the manuscript.

Reviewer: "Two further points should also be corrected: Please avoid on page 3 the term "for the first time" – in each case a novel publication should show a novel finding "for the first time", otherwise it's not worth to be printed."

Author: We appreciated this suggestion and deleted the phrase "for the first time". The corrected manuscript version is the following

Manuscript text:" …Moreover, we demonstrated that genetic suppression of FKBP51 results in the inhibition of IL1beta-driven p50/p65 nuclear translocation (i.e. NFκB pathway activation) and a subsequent reduction in the release of pro-inflammatory cytokines…."

Reviewer: "And in Fig. 1 (or is it Fig. 3?) please correct the typing error of "neutrophils"."

Author: We also apologize for the misspelling in Fig. 1. We corrected this.

Second Editorial Decision - 15-Aug-2018

Dear Dr. Kästle,

It is a pleasure to provisionally accept your manuscript entitled "Modulation of steroid sensitivity and NFκB signalling by FKBP51: A novel anti-inflammatory drug target" for publication in the European Journal of Immunology. 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: https://onlinelibrary.wiley.com/toc/15214141/0/ja). 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. Francesco Annunziato

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