IL-28A(IFN-λ2) modulates lung DC function to promote Th1 immune skewing and suppress allergic airway disease

Ourania Koltsida, Michael Hausding, Athanasios Stavropoulos, Sonja Koch, George Tzelepis, Caroline Übel, Sergei V. Kotenko, Paschalis Sideras, Hans A. Lehr, Marcus Tepe, Kevin M. Klucher, Sean E. Doyle, Markus F. Neurath, Susetta Finotto and Evangelos Andreakos

Corresponding author: Evangelos Andreakos, Biomedical Research Foundation-Academy of Athens

Review timeline:
- Submission date: 11 November 2010
- Editorial Decision: 23 December 2010
- Revision received: 03 March 2011
- Accepted: 23 March 2011

Transaction Report:
(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 23 December 2010

Thank you for the submission of your manuscript "IL-28(IFN-λ) modulates lung DC function to promote Th1 immune skewing and suppress allergic airway disease" to EMBO Molecular Medicine. We have now heard back from the three referees whom we asked to evaluate your manuscript. You will see that they find the topic of your manuscript potentially interesting. However, they also raise some concerns on the study, which should be addressed in a revision of the manuscript.

In particular, reviewer #3 highlights that possible effects of IL-28 on cytokine secretion of lung epithelial cells, which could also affect lung Th2 responses, should be investigated. Of note, reviewer #1 highlights the need to analyze lymphocyte numbers as well as IL-4 levels and to provide more detailed information about the subtype of IL-28 used in the study.

Given the positive evaluations, we would like to welcome a revision of your manuscript if you can convincingly address the issues that have been raised within the space and time constraints outlined below.

Revised manuscripts should be submitted within two months of a request for revision. They will otherwise be treated as new submissions, unless arranged otherwise with the editor.

I look forward to seeing a revised form of your manuscript as soon as possible.
Best wishes for the holidays,

Yours sincerely,

Editor
EMBO Molecular Medicine

REFEREE REPORTS:

Referee #1:

This is a very clearly presented manuscript providing interesting and novel data on the affects of IL-28 on dendritic cell function and promotion of Th1 immune responses with suppression of allergic airway disease in appropriate mouse models. The data are novel and important and in my view merit publication with minor modifications.

1. Throughout the manuscript the authors refer to IL-28 as a single gene/protein. However, as mentioned in the introduction IL-28 exists as IL-28A and IL-28B. Although these are almost identical proteins, they are actually distinct genes and the authors should refer to whichever of IL-28A or B they mean in the relevant experiments they have carried out. They should also insert a very brief reference to the fact that they have examined one or both subtypes in the discussion and discuss whether it is likely that the results would pertain to both types and also to IL-29.

2. The introduction is rather lengthy at over 3 pages. This could be shortened by approximately 1/3 or greater.

RESULTS SECTIONS - page 8

In many of the figures cellular data from the asthma model are given providing data on eosinophils and neutrophils. This is rather unusual for an asthma model as one normally expects to see eosinophils and lymphocytes or perhaps all three types. Please provide the data on lymphocyte numbers in the BAL fluid for figures 1, 2, 3, 6 and 7 or provide an explanation why lymphocytes are not increased in this asthma model.

Similarly IL-4 data is missing. As an archetypal Th2 cytokine. Please provide IL-4 data for figures 1, 2, 3, 4D, 5, 6 and 7.

Page 10 - line 5

It is stated that a marked increase in eosinophil cell infiltration was observed in figure 3A. This does not look a very marked change to me. I would suggest deleting "marked" and just stating that eosinophils were increased.

Page 11, 2nd paragraph, line 8
Similarly on page 11, in the middle of the second paragraph it is stated that IFN-λ production was severely impaired in figure 4B and 4C. Figure 4B does not look like a severe impairment. I would suggest to delete "severely".

Page 13 - first line
30 pg of IL-12 is not a high level in my view. Please delete "high levels of".

Discussion - page 16
This is also somewhat long at 5 1/2 pages. Please reduce by 20-25%.

Figure 1, diagram B
Levels of IL-28 are given below 10 pg/ml. The limits of detection for the R&D duo set used in our hands are around 30-40 pg/ml. Please either provide standard curves showing you are able to detect levels below 10 pg/ml or provide revised data for this figure using an appropriate detection limit. Please state the detection limit in the methods.

Referee #2 Comments on Novelty/Model system:

This manuscript describes the very interesting ability of IL-28 to modulate allergic lung inflammation in mice through induction of Th1 immunity. The work was conducted in a scientifically sound and exhaustive manner, and the interpretation - that IL-28 can suppress allergic lung inflammation and that there is a requirement for the Th1 pathway to observe this suppression - is novel, fully supported by the data and demonstrated using a variety of approaches. In addition, the results should be of interest to the journal's audience and to the general immunology community. I recommend that the manuscript be accepted for publication in EMBO Molecular Medicine without modification.

Referee #2:

This manuscript describes the very interesting ability of IL-28 to modulate allergic lung inflammation in mice through induction of Th1 immunity. The work was conducted in a scientifically sound and exhaustive manner, and the interpretation - that IL-28 can suppress allergic lung inflammation and that there is a requirement for the Th1 pathway to observe this suppression - is novel, fully supported by the data and demonstrated using a variety of approaches. In addition, the results should be of interest to the journal's audience and to the general immunology community.

Referee #3 Comments on Novelty/Model system:

no ethical concerns. rest of the concerns are in the comments below.

Referee #3:
The paper by Koltsida is a well written paper that shows that IL-28 can prime Th1 responses by inducing IL-12p70 and IFNγ and suppress Th2 responses. The authors also observed that IL-28 can suppress OX40L expression in DCs. Although most of the conclusions are supported there are a few alternative hypotheses that could exist.

1. The finding of reduced OX40L is only correlative. Do the authors have any data using in vitro differentiation of Th2 cells that IL-28 mediated suppression of OX40L is required for IL-28's affect on reduced Th2 priming?
2. IL-28Ra is also expressed on lung epithelial cells. To that end does IL-28 affect expression of IL-25, TSLP, or IL-33 that are also important in controlling lung Th2 immune responses?

1st Revision - Authors' Response 03 March 2011

We thank the reviewers for their comments and suggestions.

Referee #1 (Remarks to the Author):

This is a very clearly presented manuscript providing interesting and novel data on the affects of IL-28 on dendritic cell function and promotion of Th1 immune responses with suppression of allergic airway disease in appropriate mouse models. The data are novel and important and in my view merit publication with minor modifications.

1. Throughout the manuscript the authors refer to IL-28 as a single gene/protein. However, as mentioned in the introduction IL-28 exists as IL-28A and IL-28B. Although these are almost identical proteins, they are actually distinct genes and the authors should refer to whichever of IL-28A or B they mean in the relevant experiments they have carried out. They should also insert a very brief reference to the fact that they have examined one or both subtypes in the discussion and discuss whether it is likely that the results would pertain to both types and also to IL-29.

Reply: We have used throughout IL-28A. We have now amended the text and figures accordingly. We have also introduced a comment in the Discussion explaining that our data are likely to be similar to those that would be obtained after IL-28B or IL-29 application.

2. The introduction is rather lengthy at over 3 pages. This could be shortened by approximately 1/3 or greater.

Reply: We have shortened the introduction as suggested.

RESULTS SECTIONS - page 8

In many of the figures cellular data from the asthma model are given providing data on eosinophils and neutrophils. This is rather unusual for an asthma model as one normally expects to see eosinophils and lymphocytes or perhaps all three types. Please provide the data on lymphocyte numbers in the BAL fluid for figures 1, 2, 3, 6 and 7 or provide an explanation why lymphocytes are not increased in this asthma model.

Reply: In this model, eosinophils are the dominant inflammatory cell population representing 70% of the total cells infiltrating the airways. Neutrophils and lymphocytes are also increased although
they are found in much lower numbers. As we routinely assess the presence of all cell types in the BALF, we have now added these data to the manuscript.

Similarly IL-4 data is missing. As an archetypal Th2 cytokine. Please provide IL-4 data for figures 1, 2, 3, 4D, 5, 6 and 7.

**Reply:** We provide IL-4 data for CD4\(^+\) T cells isolated from the lung of OVA sensitized and challenged mice after IL-28 treatment (Figure S1A). We also provide IL-4 data for lung CD4\(^+\) T cells from IL-28Ra\(^-\) mice (Figure S2A). In Figure 4A, we further include IL-4 data from spleen cells after primary immunization with OVA in alum. In contrast, in OVA-specific T cell response assays in draining lymph nodes, we cannot pick up consistently IL-4 (ELISA detection limit ~15 pg/ml). IL-4 is probably produced in low levels in these cell culture supernatants and/or is rapidly taken up and degraded. This is in agreement with literature from other labs using this model (Schnyder-Candrian et al. JEM 2006; Phipps et al. AJRCCM 2009; Nakagome et al. JI 2009; Otero et al. Blood 2010). We have now introduced a comment for IL-4 production in the Results section.

**Page 10 - line 5**

It is stated that a marked increase in eosinophil cell infiltration was observed in figure 3A. This does not look a very marked change to me. I would suggest deleting "marked" and just stating that eosinophils were increased.

**Reply:** We have now corrected this point.

**Page 11, 2nd paragraph, line 8**

Similarly on page 11, in the middle of the second paragraph it is stated that IFN-\(\beta\) production was severely impaired in figure 4B and 4C. Figure 4B does not look like a severe impairment. I would suggest to delete "severely".

**Reply:** We have also corrected this point to accurately reflect the data.

**Page 13 - first line**

30 pg of IL-12 is not a high level in my view. Please delete "high levels of".

**Reply:** We have amended this by replacing with ‘significant’.

**Discussion - page 16**

This is also somewhat long at 5 1/2 pages. Please reduce by 20-25%.

**Reply:** We have reduced the length of the discussion.

**Figure 1, diagram B**

Levels of IL-28 are given below 10 pg/ml. The limits of detection for the R&D duo set used in our hands are around 30-40 pg/ml. Please either provide standard curves showing you are able to detect levels below 10 pg/ml or provide revised data for this figure using an appropriate detection limit. Please state the detection limit in the methods.
**Reply:** We also get a similar level of detection with the same R&D Duo Set. The difference here is that we concentrated the BALF 10X using the Amicon Ultra-15 centrifugal filter columns (Millipore, USA) with a molecular weight cut-off of 10 KDa. This enabled us to increase the detection limit in the BALF 10 times. We had a comment for that in the originally submitted Supplemental Figures. We have now moved that to the main text of the Materials & Methods section and also introduced this information in the figure legend.

**Referee #2 (Novelty/Model system Comments for Author):**

*This manuscript describes the very interesting ability of IL-28 to modulate allergic lung inflammation in mice through induction of Th1 immunity. The work was conducted in a scientifically sound and exhaustive manner, and the interpretation - that IL-28 can suppress allergic lung inflammation and that there is a requirement for the Th1 pathway to observe this suppression - is novel, fully supported by the data and demonstrated using a variety of approaches. In addition, the results should be of interest to the journal's audience and to the general immunology community. I recommend that the manuscript be accepted for publication in EMBO Molecular Medicine without modification.*

**Referee #2 (Remarks to the Author):**

*This manuscript describes the very interesting ability of IL-28 to modulate allergic lung inflammation in mice through induction of Th1 immunity. The work was conducted in a scientifically sound and exhaustive manner, and the interpretation - that IL-28 can suppress allergic lung inflammation and that there is a requirement for the Th1 pathway to observe this suppression - is novel, fully supported by the data and demonstrated using a variety of approaches. In addition, the results should be of interest to the journal's audience and to the general immunology community.*

**Referee #3 (Novelty/Model system Comments for Author):**

*no ethical concerns. rest of the concerns are in the review below.*

**Referee #3 (Remarks to the Author):**

*The paper by Koltsida is a well written paper that shows that IL-28 can prime Th1 responses by inducing IL-12p70 and IFNg and suppress Th2 responses. The authors also observed that IL-28 can suppress OX40L expression in DCs. Although most of the conclusions are supported there are a few alternative hypotheses that could exist.

1. The finding of reduced OX40L is only correlative. Do the authors have any data using in vitro differentiation of Th2 cells that IL-28 mediated suppression of OX40L is required for IL-28’s affect on reduced Th2 priming?*

**Reply:** We agree with the reviewer on that point. We have therefore introduced a comment to clearly indicate the correlative nature of this finding. Unfortunately, it is difficult to demonstrate that this is (or isn’t) important as IL-28A-treated DCs are altered in several ways.
2. IL-28Ra is also expressed on lung epithelial cells. To that end does IL-28 affect expression of IL-25, TSLP, or IL-33 that are also important in controlling lung Th2 immune responses?

Reply: We have now measured IL-25, TSLP and IL-33 in the BALF of OVA sensitized and challenged mice. We found that IL-28A treatment also inhibited the production IL-25 and TSLP, similarly to IL-13, while IL-33 was barely detectable. Although these data raise the possibility that respiratory epithelium is also affected, further studies involving the use of conditional IL-28Ra-/- or bone marrow transplantation experiments will be needed to clarify this issue. In our setting, it is still likely that inhibition of IL-25 and TSLP is a consequence of reduced Th2 responses in the lung resulting in deactivation of the respiratory epithelium. A comment for that has been added in the Discussion.

2nd Editorial Decision 23 March 2011

We are pleased to inform you that your manuscript is accepted for publication and all reviewers who have been asked to re-review the manuscript indicated that it is suitable for publication. The manuscript will be sent to our publisher to be included in the next available issue of EMBO Molecular Medicine if or once we have received your licenses (see below).

Please see below for additional IMPORTANT information and instructions regarding your article, its publication, and the production process.

Congratulations on your interesting work.

Yours sincerely,

Editor
EMBO Molecular Medicine