European Journal of Immunology

MHC class II alleles associated with Th1 rather than Th17 type immunity drive the onset of early arthritis in a rat model of rheumatoid arthritis

Jonatan Tuncel, Sabrina Haag, Rikard Holmdahl

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Review Timeline: Submission date: 12-Oct-2016
First Editorial decision: 03-Nov-2016
First Revision received: 06-Dec-2016
Accepted: 23-Dec-2016

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 - 03-Nov-2016

Dear Dr. Haag,

Manuscript ID eji.201646760 entitled "MHC class II alleles associated with Th1 type immunity drive the onset of early arthritis in a rat model of rheumatoid arthritis", 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.

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. **In particular, please edit your figure legends to follow Journal standards as outlined in the editorial comments. Failure to do this will result in delays in the re-review process.**

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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 referee(s) 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,
Laura Soto Vazquez

on behalf of Prof. Iain McInnes

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Reviewer: 1

Comments to the Author

The manuscript by Tuncel et al., seeks to better understand the T cell response in a rat model of rheumatoid arthritis. The authors convincingly argue and provide data that this model is superior to the mouse based models (used very commonly despite significant caveats) for assessing the T cell role in the establishment of arthritis. Using the Pristane rat model, the authors conduct a logical series of well controlled experiments that provide further useful information on the T cell response. The data is convincing and the interpretations consistent with the data generated. The manuscript is of broad interest to the immunology community.
Minor comments:
- In Figure 4, the authors assess cytokine production by intracellular flow cytometry and provide evidence of early skewing towards a Th1 response in the more susceptible rat strains. The differences described appear significant but modest in value. The data is more convincing when combined with the later data where IFN-γ is blocked and milder disease experienced.
The data in Figure 4 would be improved if numbers of IFN-γ+ and IL-17+ T cells were shown. The scale used in Figure 4C is done to highlight the changes between strains, but is slightly misleading given the values are so low. Numbers of cells would really complement this.

- Given the data in Figure 6 where neutralization of IL-17A blocks the late development of disease, additional data on the cytokine production at later time points (e.g. Day 16, 20, 24) would help make sense of this finding.
- On Page 16, Line 7, data is described on the effect of INFg treatment delaying the onset of arthritis. This data is described as “data not shown”. These data should be included.
- The font size in Figure 1F is overly small and needs to be increased to make the information legible

Reviewer: 2

Comments to the Author

This is an interesting study showing that a Th1-driven response including IFNg is needed to induce arthritis by pristane, but Th17 cells and IL-17 are needed to maintain the disease in genetically prone animals. The experimental design is logical, results are clear.

There is one major issue that needs to be addressed. In human RA, anti-IL-17 treatment was almost ineffective. It is likely that what is going on in the synovial tissue is not always parallel what is in the blood or lymph nodes. In humans, large numbers of Th17 cells and high IL-17 levels could be detected in the blood but probably not in the tissue. Also, Th17 cells may shift to Th1 and also to Tregs. In this study, authors performed experiments only on blood and LN cells. Therefore:
- immunohistochemistry of synovial tissues should be performed in order to determine Th1, Th17, Treg subsets in situ, as well as their relative enrichment or absence in synovium. This should also be studied following the disease course (early disease vs maintenance)
- are there Th1/Th17 and Treg/Th17 cells carrying dual phenotype that can be switched to either phenotype? (These cells have been described in human RA as well as rodent models) If so, please try to detect these cells in the synovium
- Only very few groups use the PIA model so in the Discussion please compare the findings in this model with other models. How would these results reflect CIA or human RA?

First Revision – authors’ response 06-Dec-2016

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Authors' response to Reviewers

Reviewer: 1

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Authors: We have now included data on the total number of Th1 cells in dLNs for the first 12 days of PIA in Fig. 4D. These new data show that there is a significantly larger population of Th1 cells in DA and DA.1FR61 (approx. 550.000 cells per rat) vs. DA.1HR10 and DA.1UR10 (approx. 250.000 cells per rat; $P<0.0001$ HR10 vs. DA) on day 5 after pristane-administration. The difference in the total number of Th1 cells was smaller but still significant on day 8 between DA/DA.1FR61 vs. DA.1HR10 ($P<0.05$). For comparison, we have also included data showing the total number of Th17 cells in dLNs for the same period (which, as expected, did not show a difference between the strains) to show that the increase in total Th1 cells is not just a reflection of an increase in total CD4 T cells in DA and DA.1FR61.

- Given the data in Figure 6 where neutralization of IL-17A blocks the late development of disease, additional data on the cytokine production at later time points (e.g. Day 16, 20, 24) would help make sense of this finding.

Authors: The frequency of IL-17 and IFN-γ producing T cells seems to stabilize between the strains close to the onset of overt disease (last data point is on day 12 post immunization, Figure 4C and D). Although we have not determined the frequency of Th1 and Th17 cells over the entire course of PIA, we have seen that the frequency of Th17 cells in rats with chronic disease is relatively similar to that shown for LNs in pre-arthritic rats (~0.3-0.5% of CD4 T cells), while the relative proportion of Th1 cells increases from 0.5% in early PIA to 3-4% in chronic PIA (unpublished, Fig. R1). Further, anti-IL-17 treatment in chronic PIA does not influence the abundance of Th17 or Th1 cells but has a remarkable impact on disease development (as we have also shown for early acute PIA, Fig. 6). Importantly, the arthritogenicity of LN derived T cells peaks at or even before the onset of disease (day 11), and is substantially reduced in rats with established disease (day 20; unpublished data, Fig. R2). We therefore believe that it is not meaningful to assess the abundance of Th1 and Th17 cells beyond day 12 in the dLN.

- On Page 16, Line 7, data is described on the effect of INFg treatment delaying the onset of arthritis. This data is described as 'data not shown'. These data should be included.

Authors: We have now included data for disease onset for rats treated with anti-IFN-γ and IL-17 in Fig. 5C.

- The font size in Figure 1F is overly small and needs to be increased to make the information legible

Authors: All font sizes in figures and tables have now been adjusted.

Reviewer: 2

Comments to the Author

This is an interesting study showing that a Th1-driven response including IFNg is needed to induce arthritis by pristane, but Th17 cells and IL-17 are needed to maintain the disease in genetically prone animals. The experimental design is logical, results are clear.

There is one major issue that needs to be addressed. In human RA, anti-IL-17 treatment was almost ineffective. It is likely that what is going on in the synovial tissue is not always parallel what is in the blood or lymph nodes. In humans, large numbers of Th17 cells and high IL-17 levels could be detected in the blood but probably not in the tissue. Also, Th17 cells may shift to Th1 and also to Tregs. In this study, authors performed experiments only on blood and LN cells. Therefore:
- immunohistochemistry of synovial tissues should be performed in order to determine Th1, Th17, Treg subsets in situ, as well as their relative enrichment or absence in synovium. This should also be studied following the disease course (early disease vs maintenance)
- are there Th1/Th17 and Treg/Th17 cells carrying dual phenotype that can be switched to either phenotype? (These cells have been described in human RA as well as rodent models) If so, please try to detect these cells in the synovium.

Authors: We agree with the Reviewer that the presence of Th1, Th17 and Treg cells needs to be investigated also in the synovium of rats with ongoing PIA. However, we believe that this analysis is beyond the scope of the current study, which intended to demonstrate that the initial T cell polarization is determined by the MHCII genotype. Further, while the genetic association between RA and MHCII genes is very well defined, the implications of this association on T cell priming and subsequent disease development are not yet understood. This initial phase of arthritis is for obvious reasons extremely difficult to study in humans, highlighting the usefulness of animal models and in particular models that are not driven by exogenous antigens.
We are also aware of that anti-IL-17 treatment has not been efficient in RA, while it has been extremely potent in different animal models, including acute and chronic PIA (Fig. 6, Fig. R1). In PIA, the depletion of IL-17 seems to prevent recruitment of neutrophils to the joints, since the severity of PIA strictly correlates with the number of neutrophils in the blood [1]. This suggest that the IL-17 targeted is likely not only T cell derived. Interestingly, this is not the case in the adoptive transfer model of PIA (Fig. R3, inset table) where neutrophil counts are generally much lower than in the induced model despite similar arthritis severity (Fig. R3).
We therefore believe that the suggested experiments would be more revealing if performed in both model systems in parallel. Such a comparison should be valuable to get a better

understanding of which role different T cell lineages may play during the perpetuation of arthritis. Although we agree with the reviewer, considering the extent of these experiments, we think this is not within the possible reach for the present work.

- *Only very few groups use the PIA model so in the Discussion please compare the findings in this model with other models. How would these results reflect CIA or human RA?*

Authors: In the revised version of the manuscript, we discuss new findings in CIA on the link between NETosis (which is also a prominent feature in early PIA [2]) and the development of Th1 type immune responses in dLNs as well as the impact this may have on early disease development. We also discuss new findings in humans showing that individuals with an increased risk of developing RA (that is, individuals with elevated levels of RF and ACPAs) have a higher proportion of Th1 cells in their lymph nodes whereas they have the same proportion of Th17 and Th2 cells as do individuals with no increased risk of developing RA.

References

1. **Tuncel J**, **Haag S**, **Hoffmann MH**, **Yau ACY**, **Hultqvist M**, **Olofsson P**, **Bäcklund J**, *et al.* Animal Models of Rheumatoid Arthritis (I): Pristane-Induced Arthritis in the Rat. *PLoS ONE*. 2016; **11**:e0155936.DOI: 10.1371/journal.pone.0155936.

2. **Herman S**, **Kny A**, **Schorn C**, **Pfatschbacher J**, **Niederreiter B**, **Herrmann M**, **Holmdahl R**, *et al.* Cell death and cytokine production induced by autoimmunogenic hydrocarbon oils. *Autoimmunity*. 2012; **45**:602–611.DOI: 10.3109/08916934.2012.719948.

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Second Editorial Decision - 20-Dec-2016

Dear Dr. Haag,

It is a pleasure to provisionally accept your manuscript entitled "MHC class II alleles associated with Th1 rather than Th17 type immunity drive the onset of early arthritis in a rat model of rheumatoid arthritis" 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: 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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